As pointed out above, pulmonary administration of a complement inhibitory protein is preferred for the treatment of lung disorders or diseases because of the high local concentration of complement inhibitory protein that can be delivered, the localization of significant amounts of the complement inhibitory protein in extravascular space, and the ability to limit or minimize systemic effects of the complement inhibitory protein.

It is particularly contemplated that a formulation of the present invention can be used for prophylaxis or therapy of smoke inhalation injury.

5.3.3. USE OF COMPLEMENT INHIBITORY PROTEINS FOR THE TREATMENT OF BRONCHOCONSTRICTION

As demonstrated in an example *infra*, complement inhibitory proteins of the invention can be used for the treatment of bronchoconstriction. The complement inhibitory protein can be administered systemically, and more preferably parenterally, i.e., via an intraperitoneal, intravenous, perioral, subcutaneous, intramuscular, intraarterial, etc. route, in order to treat bronchoconstriction. In a preferred embodiment, the complement inhibitory protein can be administered via the pulmonary route in order to treat bronchoconstriction. Pulmonary administration of a complement inhibitory protein is described above.

Bronchoconstriction can result from a number of conditions or disorders. These include but are not limited to asthma, especially allergic asthma, anaphylaxis, especially immune-mediated anaphylaxis, chronic obstructive pulmonary disease, and various non-specific irritants or lung insults, such as are included Table II, *supra*, under the headings "Diseases," "Chemical Injury," "Smoke Injury," "Organic Dust Diseases," "Fibrogenic Dust Diseases," "Smoke Injury" and "Thermal

Injury." It is particularly contemplated that the systemic or pulmonary administration of a complement inhibitory protein can be used for prophylactic or therapeutic treatment of bronchoconstriction resulting from smoke inhalation.

5.3.4. USE OF COMPLEMENT INHIBITORY PROTEINS FOR THE TREATMENT OF ANAPHYLAXIS

In a specific embodiment, a complement inhibitory protein can be used in the treatment of anaphylaxis, in particularly hyperimmune anaphylaxis. Anaphylaxis is a systemic immune response caused by exposure to a substance to which a subject has become hypersensitive. Such reactions are unexpected, and can be life threatening. Anaphylaxis usually occurs within minutes to hours of exposure to the antigen. Many proteins and polypeptides can produce anaphylaxis in a subject (See, e.g., Lichtenstein and Fauci, *Current Therapy in Allergy and Immunology*, B.C. Decker Inc.: Philadelphia, esp. p. 79).

In another aspect of the invention, a complement inhibitory protein can be administered prophylactically or therapeutically for the treatment of an anaphylactoid reaction or idiopathic anaphylaxis. Anaphylactoid reactions or idiopathic anaphylaxis involve nonimmunologic release of the same or similar agents as in anaphylaxis. Such reactions usually are caused by exposure to various therapeutic or diagnostic agents, such as contrast media used in radiologic examinations. Some agents known to cause anaphylactoid reactions include but are not limited to acetyl salicylic acid, non-steroidal anti-inflammatory agents, curare, narcotics, mannitol and iodinated radiopaque contrast agents.

For the prophylaxis or treatment of anaphylaxis, or anaphylactoid reactions or idiopathic anaphylaxis, the complement inhibitory protein can be administered systemically, and more preferably parenterally, i.e., via an intraperitoneal, intravenous, perioral, subcutaneous, intramuscular, intraarterial, etc. route, in order to treat anaphylaxis. In a preferred embodiment, the complement inhibitory protein can be administered via the pulmonary route in order to treat anaphylaxis, especially for the treatment of bronchoconstriction associated with anaphylaxis. In addition to bronchoconstriction, administration of a complement inhibitory protein can attenuate or prevent blood pressure changes, decrease in circulating platelet count, and shock associated with anaphylaxis. Pulmonary administration of a complement inhibitory protein is described above.

In a specific example *infra*, soluble CR1 reduces or eliminates symptoms of anaphylaxis resulting from antigen challenge of a passively or actively immunized subject. In a specific embodiment, the sCR1 is administered by i.p. and/or i.v. route.

5.4. ANIMAL MODELS FOR EVALUATING THE FORMULATIONS OF THE INVENTION

In a preferred aspect of the invention, the complement inhibitory protein or formulation of the invention is effective in inhibiting complement activity associated with anaphylaxis in the following model system. Guinea pigs are actively sensitized with ovalbumin in complete Freund's adjuvant (see Example 6, *infra*). Two groups of about seven or so animals are used. Group 1 is a control group which receives phosphate buffered saline. Group 2 is treated with a complement inhibitory protein, e.g., soluble CR1. At -1

hour, the animals are anesthetized, e.g., with pentobarbital or possibly ketamine/xylazine, and instrumented for measurement of bronchoconstriction and blood pressure. At -7 min, arterial blood samples are obtained. Samples of about 0.5 ml are appropriate. At -5 min the PBS or complement inhibitory protein (in a solution with a dispersant, e.g., a surfactant such as Tween 20), in particular sCR1, is aerosolized and administered by inhalation for about 3 min in about 3 ml volume. Aerosolization can be accomplished with a nebulizer, such as a DeVilbiss Porta Sonic Nebulizer. The compliance and resistance recorders for measuring bronchoconstriction and blood pressure should be interrupted during pulmonary administration of the aerosol formulation. At -2 min, the recording of compliance and resistance are resumed. A blood sample is obtained at -1 min. At time 0, ovalbumin is administered parenterally or, more preferably, by inhalation. For example, a 1% ovalbumin solution in Tween 20 can be nebulized for 12 sec through the pneumotachograph and pump using a DeVilbiss Model 65 Ultrasonic Nebulizer. The amount of ovalbumin (or its concentration) can be varied up or down to induce a satisfactory response. Blood samples are obtained at +2, +7 and +20 min, and compliance and resistance measured continuously. After completion of the experiment, bronchoalveolar lavage (BAL) will be collected (about 15 ml) and BAL cells will be counted. The BAL supernatant will be tested to determine complement inhibitory protein, e.g., sCR1 levels and for protein content. Blood samples are used for counts of circulating white cells and platelets. Differential blood counts can be done and hematocrit obtained. Plasma samples can be tested to determine the level of C3 conversion (a measure of complement

activation) and the level of complement inhibitory protein, e.g., sCR1.

In another embodiment, the effectiveness of complement inhibitory proteins and the formulations of the invention for the treatment of smoke inhalation injury can be tested. Many models for smoke inhalation injury are known in the art. For example, smoke can be generated by thermolysis of a fuel, e.g., polytetrafluoroethylene, in a crucible furnace at a constant temperature of 600°C with a constant airflow rate. The smoke can be mixed with oxygen and animals, e.g., rats, can be exposed to the smoke for an appropriate period of time, e.g., 20 min. Group comparisons can be made between those animals treated prophylactically or after exposure to the smoke with an effective dose of a complement inhibitory protein, such as sCR1. The sCR1 can be administered parenterally, such as is described in the example, *infra*, or by pulmonary administration.

The invention can be better understood by referring to the following example, which is provided merely by way of exemplification and is not intended to limit the invention.

6. EXAMPLE: COMPLEMENT RECEPTOR 1 (CR1) DECREASES BRONCHOCONSTRICTION IN A SENSITIZED GUINEA PIG

Along with life threatening bronchoconstriction, systemic anaphylaxis involves a serious hypotensive response often complicated by cardiac arrhythmias. Products of complement system activation are potential mediators of systemic anaphylaxis. The present example shows that the soluble complement receptor 1 (sCR1) can be used to inhibit activation of the classical and alternative pathways of complement in the guinea pig and will prevent bronchoconstriction and

changes in blood pressure induced by intravenous antigen injection in guinea pigs either passively or actively sensitized to the antigen ovalbumin.

6.1. MATERIALS AND METHODS

6.1.1. RESPIRATORY AND BLOOD PRESSURE MEASUREMENTS

Pulmonary resistance and dynamic lung compliance were measured continuously in mechanically respirated, pentobarbital-anesthetized (25 mg/kg, i.p.) male guinea pigs (Hartley guinea pigs, Harlan Sprague-Dawley, Inc., Indianapolis, IN or Sasco, Inc., Omaha, NE) as described previously (Regal and Bell, 1987, Int. Archs. Allergy Appl. Immun. 84:414-423). Tracheal airflow was measured with a Fleisch pneumotachograph and transpulmonary pressure measured via a needle inserted in the pleural cavity. Both tracheal airflow and transpulmonary pressure were fed into an on-line pulmonary mechanics computer (Model 6, Buxco Electronics, Sharon CT) which calculated pulmonary resistance and dynamic lung compliance by the method of Amdur and Mead (1958, Am. J. Physiol. 192:364-370). Mean arterial blood pressure was monitored via the femoral artery using a Statham PM23Db pressure transducer. A jugular vein was cannulated for administration of drugs and a carotid artery for blood sampling. Animals were allowed to stabilize 15 to 20 min prior to experimental manipulations. Because of tachyphylaxis, each animal received only one dose of antigen. Results are expressed as the mean ± S.E. of the percentage of change from the control compliance, resistance or blood pressure before ovalbumin (OA) or bovine serum albumin (BSA) addition. After injection of OA or BSA, data were collected for 20 min. The average response in percent change for each group of animals at each 6-second interval is plotted. For clarity, standard error bars are included only at

selected time points. For studies assessing the effect of sCR1 on the responsiveness to histamine or bradykinin, increasing doses of the agonist were given at 1-min intervals and the maximum percent change for each dose was determined.

6.1.2. ACTIVE AND PASSIVE SENSITIZATION.

Guinea pigs (200-300 g) were actively sensitized by the i.p. injection on days 0,2, and 4 of 0.4 ml of an emulsion made by mixing equal volumes of complete Freund's adjuvant with ovalbumin (5mg/ml) in normal saline solution (NSS). Actively sensitized guinea pigs were challenged with antigen intravenously on days 21-34, at which time they had attained a weight of 350-450 g.

For passive sensitization, IgG antibody to ovalbumin was obtained from pooled serum samples of guinea pigs immunized with ovalbumin, and IgG and IgE-type antibodies to ovalbumin were separated by passage over a protein A-Sepharose column as previously described (Regal, 1984, J. Pharmacol. Exp. Ther. 228:116-120). Briefly, sera containing both IgG and IgE antibody to ovalbumin were passed over a protein A-Sepharose column. The antibody, plus other serum components that passed through the column, was characterized as IgE-type antibody by its heat lability in passive cutaneous anaphylaxis and its persistence in guinea pig skin for at least 14 days. The cytophilic antibody which was bound to the column was characterized as IgG by its heat stability in passive cutaneous anaphylaxis and its lack of persistence in guinea pig skin. The IgG fraction was a combination of IgG_1 and IgG_2 (Regal, 1984, *supra*).

IgG antibody was dialyzed against saline and stored in aliquots at - 70° C for later use. Passive sensitization of the guinea pigs was achieved by

intracardiac injection of 1.6 mg/kg IgG under ether anesthesia 12-24 hrs before the experiment in guinea pigs weighing 225-300 g.

6.1.3. MEASUREMENT OF C3 CONVERSION

C3 conversion was assessed using immunofixation techniques as described by Strong and Watkins (1979, J. Immunol. Methods 29:293-297). Plasma samples (1 ul) were applied to precut loading slits on Agarose Universal Electrophoresis film (Corning Medical, Palo Alto, Calif., USA) and electrophoresed for 90 min at 30 mA per film using Corning Universal barbital buffer containing EDTA. After electrophoresis, the film was overlaid with cellulose acetate strips soaked in the IgG fraction of goat anti-guinea pig C3 (Cooper Biomedical, East Chester, Pa., USA) and incubated at room temperature for 1 h. The film was then washed in normal saline solution, pressed, dried, and stained with Coomassie blue. A sample of yeast activated complement (YAC) was included on each gel to serve as a positive control, i.e., a sample with known C3 conversion. YAC was prepared as follows: Baker's yeast was first heat inactivated by boiling at 250 mg/ml in NSS for 30 min, and then incubated at 25 mg/ml with normal guinea pig serum at 37°C for 60 min. The yeast was removed by centrifugation at 12,000 x g for 45 min and the supernatant (YAC) aliquoted and stored at -70°C.

6.1.4. DETERMINATION OF SCR1 PLASMA LEVELS

Concentrations of sCR1 in plasma samples were quantitated by a double polyclonal bead enzyme immunoassay as previously described (Mulligan et al, 1992, J. Immunol. 148:1479-1485).

6.1.5. QUANTIFICATION OF PERIPHERAL BLOOD CELLS

Arterial blood samples were collected into ethylenediamine tetra-acetic acid (EDTA) coated tubes. Total white blood cells and platelets were counted using a hemocytometer by standard procedures.

6.1.6. MATERIALS

Histamine dihydrochloride, ovalbumin (Grade V), bovine serum albumin (Fraction V) and the acetate salt of bradykinin were obtained from Sigma Chemical (St. Louis, MO). Soluble complement receptor 1 (sCR1) containing LHRs A, B, C and D and SCRs 29 and 30, but lacking the transmembrane and cytoplasmic domains, has been described *supra* (Section 5.1). The sCR1 was prepared at a concentration of 5.96 or 5.08 mg/ml in phosphate buffered saline (PBS). sCR1 was prepared as previously described (Weisman et al, 1990, Science 249:146-151) using recombinant techniques, and contained less than 0.24 endotoxin units/ml as determined by the Limulus assay.

6.1.7. EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

Four different experimental groups were addressed in this study: 1) passively sensitized guinea pigs receiving sCR1 at a dose of 15 mg/kg i.v. 2 min before challenge with 176 μg/kg ovalbumin; 2) actively sensitized guinea pigs receiving sCR1 at a dose of 15 mg/kg i.v. 2 min before challenge with 300 μg/kg ovalbumin; 3) actively sensitized guinea pigs receiving a cumulative dose of 105 mg/kg sCR1 i.p. and i.v. before challenge with 2 mg/kg of ovalbumin; and 4) actively sensitized guinea pigs receiving a cumulative dose of 105 mg/kg sCR1 i.p. and i.v. before challenge with 2 mg/kg bovine serum albumin (BSA). The dosing regimen for animals receiving a cumulative dose of 105 mg/kg sCR1 was as follows: 24 hours prior to antigen or BSA challenge, 60 mg/kg sCR1 or 10.1 ml/kg PBS intraperitoneally; 5

minutes prior to antigen, 20 mg/kg sCR1 or 3.3 ml/kg PBS intravenously. In all 4 experimental groups, arterial blood samples were taken 1 or 2 min before i.v. administration of sCR1 or PBS, as well as 1 min before challenge with antigen or BSA. Either 2 or 3 arterial blood samples were also taken after antigen challenge for the assessment of C3 conversion, sCR1 plasma concentrations, and quantification of peripheral blood cells.

For determining differences in the time course of the percent change in compliance, resistance or blood pressure, the two tailed *t* test employed is Satterthwaites' approximation (Snedecor and Cochran, *Statistical Methods, ed. 7*, Iowa State University Press: Ames, Iowa, 1980) which does not assume equal variances. Repeated-measures analysis of variance was employed to determine if sCR1 affected the response to histamine or bradykinin as well as to determine if plasma levels of sCR1 were different in OA versus BSA challenged guinea pigs. To determine if sCR1 significantly affected the OA-induced changes in circulating cells, a two-tailed paired Student's *t* test was employed using log transformed values to stabilize variances. All tests used p=0.05 as the level of significance.

6.2. RESULTS

6.2.1. EFFECT OF 15 MG/KG SCR1 ON THE RESPONSE TO OA IN ACTIVE AND PASSIVE SENSITIZATION

The effect of a single intravenous injection of sCR1 (15 mg/kg) 2 min prior to OA challenge in both actively and passively sensitized guinea pigs was assessed (Figure 1). OA challenge of passively sensitized guinea pigs (Experimental Group 1) resulted in a large increase in resistance and a large decrease in compliance. A transient hypertensive phase was followed

by a precipitous drop in blood pressure. By 20 min blood samples could only be obtained by cardiac puncture. The administration of 15 mg/kg sCR1 did not significantly affect the response to antigen in passively sensitized guinea pigs.

A larger dose of ovalbumin was used in actively sensitized guinea pigs (Experimental Group 2) to insure that the compliance and resistance changes were as large as that seen with the passively sensitized guinea pigs. OA challenge of the actively sensitized guinea pig resulted in a bronchoconstriction similar in magnitude to that seen in the passively sensitized guinea pig. However, the blood pressure response was not as dramatic. The transient hypertensive phase was followed by only a moderate decrease in blood pressure. Treatment with 15 mg/kg sCR1 i.v. in the actively sensitized guinea pigs resulted in a very minor reduction in the OA-induced decrease in compliance, a marked shortening of the hypertensive phase of the blood pressure response and no hypotensive response. Soluble CR1 treatment did not significantly alter the baseline compliance, resistance or blood pressure compared to the PBS treated animals.

OA challenge of a passively sensitized guinea pig results in a precipitous drop in circulating white blood cells with minimal changes in circulating platelets accompanying the dramatic cardiovascular/pulmonary changes (Figure 2). Soluble CR1 treatment did not alter the OA-induced changes in circulating cells in passively sensitized animals. In the actively sensitized guinea pig, OA-challenge was accompanied by a dramatic decrease in both circulating white blood cells and platelets. Soluble CR1 treatment significantly attenuated the OA-induced decrease in circulating platelets in the actively sensitized guinea pig, suggesting that this reduction in circulating platelets was dependent on complement

activation. Soluble CR1 treatment did not significantly affect the baseline numbers of circulating white blood cells or platelets as determined by comparing cell counts before sCR1 treatment (-5 min) to those immediately before OA challenge (-1 min).

6.2.2. EFFECT A CUMULATIVE DOSE OF SCR1 ON THE RESPONSE TO OA, BSA, HISTAMINE, AND BRADYKININ IN ACTIVELY SENSITIZED GUINEA PIGS

Since our initial experiments with actively sensitized guinea pigs had demonstrated that sCR1 treatment shortened the hypertensive response to OA challenge, our continued experiments employed a higher dose of sCR1 administered over a 24 hour period prior to OA challenge. Preliminary studies in the rat had suggested that multiple dosing of sCR1 at higher doses would result in extravascular distribution of the molecule. Thus the response to OA challenge was assessed in actively sensitized guinea pigs, which had received a cumulative dose of 105 mg/kg sCR1 i.p. and i.v. over a 24 hour period prior to OA challenge (Experimental Group 3). These actively sensitized guinea pigs were challenged with a higher dose of OA to insure that a large hypotensive response to antigen would also occur. As seen in Figure 3, sCR1 significantly inhibited the OA-induced decrease in compliance and increase in resistance. The hypertensive response to OA was shortened and the hypotensive response eliminated. Additionally, OA challenge was lethal in 5 of the 9 PBS treated animals, whereas all 9 of the sCR1 treated animals survived the 20 min course of the experiment.

A group of actively sensitized guinea pigs pretreated with either PBS or sCR1 received BSA challenge as a control (Experimental Group 4). Soluble CR1 administered intravenously at -5 min in Experimental

Group 3 and 4 did not significantly alter the baseline compliance, resistance, blood pressure, circulating white blood cells or platelets when compared to changes occurring in the PBS treated controls. Initial values of compliance, resistance, blood pressure, circulating white blood cells, and platelets at the time of OA or BSA addition were also not different. After BSA challenge, compliance and resistance changes were minimal, with less than a ± 5% fluctuation from the baseline over the 27 min time period monitored after PBS or sCR1 administration i.v. (data not shown). Fluctuations in blood pressure in BSA challenged animals were more pronounced (Figure 4). Actively sensitized guinea pigs treated with a cumulative dose of 105 mg/kg sCR1 or the control PBS injections and then challenged with BSA experienced an increase in blood pressure over the 20 min period monitored after BSA challenge. This slow rise in blood pressure is indistinguishable from that observed in sCR1 treated animals challenged with OA. The sCR1 treated animals challenged with OA also showed the transient hypertensive phase characteristic of the intravenous antigen challenge in the guinea pig. The slow rise in blood pressure also occurred in animals treated with PBS and challenged with BSA. Thus, this slow rise in blood pressure was not an effect of the sCR1 itself.

The effect of a cumulative dose of 105 mg/kg sCR1 on the OA and BSA-induced changes in circulating cells was also investigated (Experimental Groups 3 and 4). As seen in Figure 5, BSA challenge of PBS or sCR1 treated animals showed minimal changes in circulating cell numbers. However, challenge of actively sensitized guinea pigs with 2 mg/kg OA resulted in a precipitous drop in both circulating white blood cells and platelets. Soluble CR1 treatment significantly inhibited the decrease in the circulating platelets at all time points

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01405

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

Databases: USPTO-APS, Medline, SciSearch, Biosis, Derwent WPI, Pascal, Life Sciences Coll., CAS, {US} Federal Research in Progress
Search terms: CR1, SCR1; complement (receptor); inhal?, (broncho)pulmonary

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-39, 45, and 52, drawn to aerosol formulations of sCR1 and to methods of using the compositions.

II. Claims 40-44 and 46, drawn to methods for treating bronchoconstriction.

III. Claims 47-51, drawn to methods of treating anaphylaxis or anaphylactoid reactions.

and it considers that the International Application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below:

The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept. The special technical feature of Group I which defines an advance over the prior art is the administration of complement inhibitory proteins in an aerosol formulation. This special technical feature is not shared by the invention of either groups II or III, the special technical features of which are, respectively, the use of complement inhibitory proteins for the treatment of bronchoconstriction and the use of complement inhibitory proteins for the treatment of anaphylaxis. The feature which defines invention I, viz., the route of administration, is not required for the practice of the invention as defined by either of Groups II and III.

Groups II and III define distinct special technical features. They are directed to methods of treating diverse conditions and symptoms, and neither method as claimed requires the other for its practice.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proceedings of the National Academy of Sciences of the USA, Volume 86, issued January 1989, R. C. Hubbard et al., "Fate of aerosolized recombinant DNA-produced α1-antitrypsin: Use of the epithelial surface of the lower respiratory tract to administer proteins of therapeutic importance", pages 680-684, see the entire document.	1-39, 45, 52
Y	US, A, 5,011,678 (WANG et al.) 30 April 1991, cols. 1-4.	1-39, 45, 52
Y	US, A, 5,077,286 (BISSOLINO et al.) 31 December 1991, col. 26: lines 32-34, col. 27: lines 1-23, col. 28: lines 14-15.	1-8, 16-39, 45, 52
Y	Complement and Inflammation, Volume 8, Number 5-6, issued 1991, S.-E. Svehag, "Adverse Effects of Clinical Intervention on the Complement System", pages 359-369, especially pages 359, 360, and 366, col. 2, 367, col. 1.	47-52
A	Blood Reviews, Volume 3, Number 2, issued June 1989, E. R. Holme et al., "Complement and Related Clinical Disorders", pages 120-129.	1-52

after OA challenge and the decrease in WBC at 2 and 7 min after OA challenge. The higher dose of 105 mg/kg sCR1 did not inhibit the OA-induced decrease in platelets to a greater extent than 15 mg/kg sCR1 i.v. (Figure 2).

In Experimental Group 4, after the response to BSA was monitored, guinea pigs were challenged with histamine, hyperinflated to return compliance and resistance to baseline values and then challenged with bradykinin. As seen in Figure 6, the bronchoconstrictor response to histamine was not affected by sCR1 treatment. Histamine also caused a 20 to 30% decrease in blood pressure which was unaffected by sCR1 pretreatment (data not shown). The effect of sCR1 treatment on the response to bradykinin was also evaluated since bradykinin produces a more pronounced decrease in blood pressure compared to histamine. Soluble CR1 treatment did not significantly affect the decrease in blood pressure induced by two successive doses of bradykinin (Figure 7). Bradykinin also caused a significant bronchoconstriction at these doses and the decrease in compliance and increase in resistance was not significantly affected by sCR1 (data not shown).

6.2.3. C3 CONVERSION

Soluble CR1 clearly inhibited the OA-induced bronchoconstriction and hypotension, suggesting that complement activation was an essential step in these events. To determine if complement system activation could be demonstrated after OA challenge, we assessed the presence or absence of detectable C3 conversion. In the process of complement activation, the complement component C3 is cleaved into C3a (9.1 kDA) and C3b (180 kDa) fragments by the enzyme C3 convertase. The cleavage product C3b is then further degraded by enzymatic action to fragments such as C3bi, C3c, and C3dg. If serum

samples from an animal are electrophoresed to separate the intact C3 molecule from its cleavage products C3b, C3bi, etc., and then probed with an antibody to guinea pig C3, two major bands are revealed: the intact C3 molecule and a broader band consisting of various C3 cleavage products) (Strong and Watkins, 1979, J. Immunol. Methods 29:293-297). In this way, an estimate of 'C3 conversion' or cleavage of the C3 molecule indicating complement activation can be obtained. Results from one PBS and one sCR1 treated animal is shown in Figure 8. Challenge of an actively sensitized guinea pig with 2 mg/kg OA (Experimental Group 3) resulted in detectable C3 conversion at all three time points examined in a PBS pretreated animal (2, 7, and 20 min after OA). Six of the 7 PBS treated animals examined had evidence of C3 conversion at all time points after OA challenge as compared to 0 of 7 sCR1 treated animals. No C3 conversion was detectable prior to OA challenge in either the PBS or sCR1 treated guinea pigs or after OA challenge in the sCR1 treated animals.

C3 conversion was also assessed in actively sensitized guinea pigs treated with 15 mg/kg sCR1 and challenged with 300 μg/kg OA (Experimental Group 2). In these animals, no C3 conversion could be detected 2 min after OA challenge but C3 conversion was clearly evident 20 min after OA challenge in all 5 PBS treated animals and in zero of 5 sCR1 treated animals. Thus, OA challenge of actively sensitized guinea pigs is accompanied by activation of the complement system as assessed by C3 conversion. No C3 conversion was detected at any time points in BSA challenged animals treated with either PBS or sCR1 (Experimental Group 4).

6.2.4. PLASMA CONCENTRATIONS OF sCR1

In all experimental groups, animals pretreated with PBS had no detectable sCR1 in the plasma. Soluble CR1 levels of guinea pigs treated with 15 mg/kg i.v. sCR1 (Experimental Group 1 and 2) are shown in Table III. Comparable plasma concentrations of sCR1 were attained in both passively and actively sensitized guinea pigs. However, the hypertensive response was only affected in actively sensitized guinea pigs.

Plasma concentrations of sCR1 in guinea pigs that received a cumulative dose of 105 mg/kg sCR1 are shown in Figure 9. Soluble CR1 concentrations did not significantly differ as determined by repeated measures ANOVA in OA vs BSA challenged animals. Plasma concentrations of sCR1 were compared at 2 and 20 min after OA challenge in actively sensitized guinea pigs treated with either 15 mg/kg i.v. sCR1 (Experimental Group 2, Table III) or 105 mg/kg sCR1 (Experimental Group 3, Figure 9). Plasma concentrations of sCR1 were significantly different at 20 min after OA challenge but not 2 min after OA challenge.

Table III.

Plasma concentrations of sCR1 in actively and passively sensitized guinea pigs. 15 mg/kg sCR1 was administered i.v. at -2 min and guinea pigs were challenged with OA at time 0 (Experimental Groups 1 and 2). Values are the mean +/- S.E. of 4 to 5 experiments.

Sensitization	Conc. of sCR1 (μg/ml) at time			
	-5 min	2 min	5 min	20 min
Passive	N.D.	--	254.2±37.5	265.7±29.4
Active	N.D.	354.4±10.3	--	239.7±22.2
N.D. = Not Detectable				

6.3. DISCUSSION

The results of this study clearly demonstrate the effectiveness of a complement inhibitory protein in reducing antigen-induced anaphylaxis. In particular, sCR1 has been shown to ameliorate or prevent many of the effects of anaphylaxis, including bronchoconstriction, blood pressure drop, and circulating platelet decrease. Furthermore, as explored more fully below, the present work is the first study to definitively implicate the complement system in anaphylaxis.

Anaphylaxis involves both serious respiratory and cardiovascular consequences. Along with life threatening bronchoconstriction, systemic anaphylaxis involves a serious hypotensive response. Knowledge of the sequence of events leading to the bronchoconstriction and hypotension is important in designing rational therapeutic regimens for the treatment of anaphylaxis. Our studies have demonstrated that inhibiting complement system activation using the molecule sCR1 will attenuate the bronchoconstrictor response as well as prevent the hypotension induced by antigen in an actively sensitized

guinea pig model of anaphylaxis. These results indicate that complement system activation contributes to the bronchoconstrictor response and is essential for the hypotensive response. In addition, the studies have demonstrated that the anaphylactic response is accompanied by complement activation with a time course consistent with a role for complement system activation in the antigen-induced events.

The sCR1 molecule has been successfully used in the present Example to minimize the symptoms of anaphylaxis, and particularly, bronchoconstriction. Soluble CR1 prevents complement activation by reversibly binding to the C3b and C4b subunits of the C3 and C5 convertase enzyme complexes which are responsible for the cleavage of C3 and C5 and the continuation of the process of complement system activation. With binding, sCR1 displaces the catalytic subunits of the C3 and C5 convertases as well as causes the proteolytic inactivation of C3b and C4b by the plasma protease Factor 1.

Inhibition of symptoms of anaphylaxis by sCR1 provides evidence that complement activation is important in antigen-induced events. Activation of the complement system produces many biologically active products (Goldstein, 1992, *supra*) which could be involved, including opsonic fragments of C3, the anaphylatoxins (C3a, C4a, C5a), the leukocytosis promoting factor C3e, fragments of Factor B, and the Membrane Attack Complex C5b-9. The anaphylatoxins C3a/C5a are known to mimic the symptoms of anaphylaxis when injected into a guinea pig. Thus, they are potentially relevant products of complement system activation to mediate the antigen-induced bronchoconstriction and changes in blood pressure. However, another result of complement system activation, the Membrane Attack Complex, also stimulates

metabolism of arachidonic acid (Morgan, 1989, Biochem. J. 264:1-14), a possible source of biologically active substances which could mediate the anaphylactic response.

These studies have shown that sCR1 does not alter the ability of the cardiovascular and respiratory systems to respond to histamine or bradykinin indicating it is not generally inhibiting cardiovascular and respiratory reactivity.

These studies have also demonstrated that C3 conversion occurred in the actively sensitized guinea pigs after antigen challenge. C3 conversion is a more sensitive indicator of complement activation than a measurement of total hemolytic complement activity, but is still far less sensitive than the measurement of C3a or C5a generation. Significant complement activation could be occurring even though C3 conversion is not detectable. Nonetheless, our studies have demonstrated the presence of C3 conversion as early as 2 min after antigen challenge when serious bronchoconstriction and blood pressure changes are occurring.

These studies have examined the role of complement system activation in two different models of guinea pig anaphylaxis. In one model (Experimental Group 1) the guinea pig was passively sensitized with a combination of IgG1 and IgG2 antibody to ovalbumin. In the other model, the guinea pigs were actively sensitized to ovalbumin using complete Freund's adjuvant. Studies of Richerson (1972, J. Lab. Clin. Med. 79:745-757) have demonstrated that sensitization with ovalbumin and complete Freund's adjuvant will result in the production of both IgG1 and IgG2 antibody to ovalbumin, whereas sensitization with low dose ovalbumin alone will result in the production of primarily IgG1 antibody to ovalbumin. Studies of Cheng, et al. (1987, Fed. Proc. 46:931) have indicated that active sensitization results

in higher circulating concentrations of IgG than passive sensitization. This is predictable since the total amount of IgG antibody injected during passive sensitization in our studies represents the amount of IgG in less than 0.5 ml of serum from a hyperimmunized animal. The dose of antigen required to generate a similar physiological response differed in the two models. Dose response curves were not generated because of tachyphylaxis, i.e. animals become unresponsive to antigen challenge after a single administration of antigen. Regardless of passive versus active sensitization, antigen challenge in either guinea pig model resulted in an intense bronchoconstriction and a transient hypertension followed by hypotension.

The effect of antigen on circulating cell populations also differed in the two different models of guinea pig anaphylaxis. In passively sensitized guinea pigs, antigen challenge did not result in significant changes in circulating platelets. Clearly, in this model, antigen-induced bronchoconstriction and changes in blood pressure occurred independently of an effect on circulating platelet numbers. In contrast, in the actively sensitized guinea pig, antigen challenge resulted in a decrease in the number of circulating platelets as well as white blood cells. Soluble CR1 treatment significantly shortened the antigen-induced hypertensive phase as well as antigen-induced decrease in circulating platelets. The dramatic effect on platelet changes indicated that sCR1 levels in the plasma were sufficient to have an effect at this site.

In the initial studies with a single i.v. treatment with sCR1, the antigen-induced response was slightly less than that in the PBS treated animals, though the effect was not significant. Thus, studies were initiated using higher doses of sCRI administered

over a 24 hour period prior to antigen challenge. Similar plasma sCR1 levels were apparent at the time of antigen challenge whether animals were dosed with a single i.v. dose of 15 mg/kg or a cumulative dose of 105 mg/kg. However, in the case of a cumulative dosing with 105 mg/kg, the antigen-induced decrease in compliance and increase in resistance was clearly inhibited and the hypotensive response to antigen was nonexistent. Our control studies also demonstrated that the cumulative dose of 105 mg/kg sCR1 did not inhibit the bronchoconstrictor response or drop in blood pressure induced by the exogenous administration of histamine or bradykinin. Thus, sCR1 was not acting nonspecifically to alter the cardiovascular/ respiratory responses in the guinea pig at these doses. These studies also suggest that the important complement activation is occurring at extravascular sites. These extravascular sites are particularly attractive targets for direct pulmonary administration of sCR1, e.g., via inhalation.

These studies are the first to demonstrate convincing evidence that complement activation is an essential step in the antigen-induced bronchoconstriction and changes in blood pressure in an actively sensitized guinea pig model of anaphylaxis. Clearly, complement activation is occurring and interference with the activation attenuates the antigen-induced events. The study also reinforces the notion that the mechanism of anaphylaxis will vary significantly depending on the model system employed. Thus, continued studies of the differing mechanisms and mediators of anaphylaxis are of importance and the complement system clearly warrants consideration as a source of those mediators.

7. EXAMPLE: AEROSOL ADMINISTRATION OF SOLUBLE COMPLEMENT RECEPTOR 1 (sCR1) IN GUINEA PIG MODELS

7.1 MATERIALS AND METHODS

Guinea pigs (200-300 g) were actively sensitized by an i.p. injection of ovalbumin (1 mg) in Freund's adjuvant on days 0, 2, and 4. On day 21, sCR1 was administered to the sensitized guinea pigs by inhalation following nebulization (exposure to a nebulized 5 mg/ml solution of sCR1 or saline for 7-14 minutes via a tracheal tube). Thirty minutes after the beginning of sCR1 administration the guinea pigs were challenged by inhalation with nebulized OA (2.5% solution for 1 minute). Pulmonary resistance, dynamic lung compliance and mean arterial blood pressure were measured continuously as described supra at section 6.1.1. Total WBC and platelets were counted in arterial blood samples. The sCR1 treatment showed an effect on resistance and blood pressure.

7.1.1 RESPIRATORY AND BLOOD PRESSURE MEASUREMENTS

Pulmonary resistance, dynamic lung compliance and mean arterial blood pressure were measured continuously as described supra at section 6.1.1. Animals were allowed to stabilize 15 to 20 min prior to experimental manipulations. Results are expressed as the mean +/- S.E. of the percentage of change from the control compliance, resistance or blood pressure before ovalbumin (OA) addition. After inhalation OA, data were collected for 20 min.

7.1.2 ACTIVE SENSITIZATION

Guinea pigs (200-300 g) were actively sensitized by the i.p. injection on days 0, 2, and 4 of 0.4 ml of an emulsion made by mixing equal volumes of

complete Freund's adjuvant with ovalbumin (5 mg/ml) in normal saline solution (NSS). Actively sensitized guinea pigs were challenged with antigen by inhalation on Day 21. The guinea pigs inhaled a nebulized 2.5% solution of OA for 1 minute. The material was nebulized in an ultrasonic nebulizer (DeVillebiss Porta-Sonic, Somerset, PA).

7.1.3 QUANTIFICATION OF PERIPHERAL BLOOD CELLS

Arterial blood samples were collected into ethylenediamine tetra-acetic acid (EDTA) coated tubes. Total white blood cells and platelets were counted using a hemocytometer by standard procedures.

7.1.4 MATERIALS

Ovalbumin (Grade V) was obtained from Sigma Chemical (St. Louis, MO). Soluble complement receptor 1 (sCR1) containing LHRs A, B, C and D and SCRs 29 and 30, but lacking the transmembrane and cytoplasmic domains, has been described *supra* (Section 5.1). The sCR1 was prepared at a concentration of 5.08 mg/ml in phosphate buffered saline (PBS). sCR1 was prepared as previously described (Weisman et al., 1990, Science 249:146-151) using recombinant techniques, and contained less than 0.254 endotoxin units/ml as determined by the Limulus assay.

7.1.5 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

Two experimental groups were used in the study. One group received sCR1 by aerosol and the other received saline thirty minutes before OA challenge. The guinea pigs were exposed to a nebulized 5 mg/ml solution of sCR1 or sterile saline for 10 minutes.

In both experimental groups, arterial blood samples were taken before aerosol administration of sCR1 or PBS, as well as 1 min before challenge with OA. Either 2 or 3 arterial blood samples were also taken after antigen challenge for the quantification of peripheral blood cells.

For determining differences in the time course of the percent change in compliance, resistance or blood pressure, the two tailed *t* test employed is Satterthwaites' approximation (Snedecor and Cochran, *Statistical Methods, ed.* 7, Iowa State University Press: Ames, Iowa, 1980) which does not assume equal variances. To determine if sCR1 significantly affected the OA-induced changes in circulating cells, a two-tailed paired Student's *t* test was employed using log transformed values to stabilize variances. All tests used p=0.05 as the level of significance.

7.2 RESULTS

OA challenge of actively sensitized guinea pigs resulted in a large increase in resistance and a large decrease in compliance. A transient hypertensive phase was followed by a precipitous drop in blood pressure. The administration of sCR1 by aerosol lowered the increase in pulmonary resistance and it reduced the severity of the hypotensive phase. OA challenge by aerosol of actively sensitized guinea pigs results in a precipitous drop in circulating white blood cells and a decrease in circulating platelets. Aerosol sCR1 treatment did not alter the OA-induced changes in circulating cells or platelets.

8. EXAMPLE: TISSUE LOCALIZATION OF sCR1 FOLLOWING INHALATION

The tissue localization of sCR1 was studied in guinea pigs following inhalation of a nebulized saline solution of sCR1 (5 mg/ml) for 7 minutes. Control animals inhaled nebulized saline. The sCR1 was visualized by immunohistochemistry using a rabbit polyclonal anti-sCR1 antibody on formalin fixed paraffin sections. The sCR1 was present throughout the lung space and was deposited on the surface of the trachea, bronchi, bronchioles, alveolar ducts and terminal alveoli.

8.1 MATERIALS AND METHODS

8.1.1 sCR1 ADMINISTRATION

Mechanically respirated, anesthetized (ketamine 30 mg/kg i.m. xylazine 2.5 mg/kg i.m.) male guinea pigs (Hartley guinea pigs, Harlan Sprague-Dawley, Inc., Indianapolis, IN or Sasco, Inc., Omaha, NE) were administered a nebulized saline solution of sCR1 (5 mg/ml) for 7 minutes by inhalation. Control animals inhaled nebulized saline. The animals were euthanized and the lungs were preserved in formalin.

8.1.2 IMMUNOPEROXIDASE STAINING PROCEDURES

Formalin fixed lung tissue was deparaffinized and rehydrated. The sections were stained for sCR1 using a rabbit anti-sCR1 antisera (T Cell Sciences, Inc., Cambridge, MA) and a VECTASTAIN Elite ABC kit (Vector Labs, Burlingame, CA). The primary antibody was used at a dilution of 1:300. The sections were counter stained by incubation in 1% (w/v) Methyl Green in methanol for 0.5 - 2 minutes.

8.2 RESULTS

The sCR1 was present throughout the lung space and was deposited on the surface of the trachea, bronchi, bronchioles, alveolar ducts and terminal alveoli. In

the figures the sCR1 stains black and the counterstained tissue appears gray. Figures 10 A and B show cross sections of a guinea pig trachea from a control animal following inhalation of nebulized saline solution (10 A), or an experimental animal following inhalation of a nebulized saline solution containing 5 mg/ml sCR1 (10 B) for 7 minutes. Figure 10 demonstrates that sCR1 is localized in the tracheal mucosa following inhalation and appears as a black stain on a gray background.

Figures 11 A and B show cross sections of a guinea pig lung from a control animal following inhalation of nebulized saline solution (11 A) or an experimental animal following inhalation of a nebulized saline solution containing 5 mg/ml sCR1 (11 B) for 7 minutes. sCR1 was visualized by immunohistochemical staining using a rabbit polyclonal anti-sCR1 antibody. In Figure 11 B, sCR1 appears as a black stain on a gray background. sCR1 was present throughout the lung and was deposited on bronchi and bronchioli, alveolar ducts and terminal alveoli. Figure 11 A shows no sCR1 in the same areas.

The present invention is not to be limited in scope by the specific embodiments describe herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. An aerosol formulation comprising a complement inhibitory protein and a pharmaceutically acceptable dispersant.

2. The aerosol formulation of claim 1 in which the complement inhibitory protein is complement receptor 1, or a fragment, derivative or analog thereof.

3. The aerosol formulation of claim 2 in which the complement receptor 1 is soluble complement receptor 1.

4. The aerosol formulation of claim 3 in which the soluble complement receptor 1 has the characteristics of the protein expressed by a Chinese hamster ovary cell DUX B11 carrying plasmid pBSCR1c/pTCSgpt as deposited with the ATCC and assigned accession number CRL 10052.

5. The aerosol formulation of claim 1 in which the dispersant is a surfactant.

6. The aerosol formulation of claim 5 in which the surfactant is selected from the group consisting of polyoxyethylene fatty acid esters, polyoxyethylene fatty acid alcohols, and polyoxyethylene sorbitan fatty acid esters.

7. The aerosol formulation of claim 6 in which the surfactant is polyoxyethylene sorbitan monooleate.

8. The aerosol formulation of claim 5 in which the concentration of the surfactant is about 0.001% to about 4% by weight of the formulation.

9. The aerosol formulation of claim 1 which is a dry powder aerosol formulation in which the complement inhibitory protein is present as a finely divided powder.

10. The dry powder aerosol formulation of claim 9 in which the complement inhibitory protein powder is lyophilized complement inhibitory protein.

11. The dry powder aerosol formulation of claim 9 which further comprises a bulking agent.

12. The dry powder aerosol formulation of claim 11 in which the bulking agent is selected from the group consisting of lactose, sorbitol, sucrose and mannitol.

13. The dry powder aerosol formulation of claim 11 in which the complement inhibitory protein is complement receptor 1, or a fragment, derivative or analog thereof.

14. The dry powder aerosol formulation of claim 13 in which the complement receptor 1 is soluble complement receptor 1.

15. The dry powder aerosol formulation of claim 14 in which the soluble complement receptor 1 has the characteristics of the protein expressed by a Chinese hamster ovary cell DUX B11 carrying plasmid

pBSCR1c/pTCSgpt as deposited with the ATCC and assigned accession number CRL 10052.

16. The aerosol formulation of claim 1 which is a liquid aerosol formulation further comprising a pharmaceutically acceptable diluent.

17. The liquid aerosol formulation of claim 16 in which the diluent is selected from the group consisting of sterile water, saline, buffered saline and dextrose solution.

18. The liquid aerosol formulation of claim 17 in which the diluent is phosphate buffered saline in the pH 7.0 to 8.0 range.

19. The liquid aerosol formulation of claim 18 in which the complement inhibitory protein is complement receptor 1, or a fragment, derivative or analog thereof.

20. The liquid aerosol formulation of claim 19 in which the complement receptor 1 is soluble complement receptor 1.

21. The liquid aerosol formulation of claim 20 in which the soluble complement receptor 1 has the characteristics of the protein expressed by a Chinese hamster ovary cell DUX B11 carrying plasmid pBSCR1c/pTCSgpt as deposited with the ATCC and assigned accession number CRL 10052.

22. A method for treating a disease or disorder involving complement comprising pulmonary administration of an amount of a complement inhibitory protein effective to inhibit complement activity to a

subject suffering from a disease or disorder involving complement.

23. The method according to claim 22 in which the complement inhibitory protein is complement receptor 1, or a fragment, derivative or analog thereof.

24. The method according to claim 23 in which the complement receptor 1 is soluble complement receptor 1.

25. The method according to claim 24 in which the soluble complement receptor 1 has the characteristics of the protein expressed by a Chinese hamster ovary cell DUX B11 carrying plasmid pBSCR1c/pTCSgpt as deposited with the ATCC and assigned accession number CRL 10052.

26. The method according to claim 22 in which the disease or disorder involving complement is selected from the group consisting of neurological disorders, disorders of inappropriate or undesirable complement activation, inflammatory disorders, post-ischemic reperfusion conditions, infectious disease, sepsis, immune complex disorders and autoimmune disease.

27. The method according to claim 22 in which the disease or disorder involving complement is a disorder of inappropriate or undesirable complement activation selected from the group consisting of hemodialysis complications, hyperacute allograft rejection, xenograft rejection and interleukin-2 induced toxicity during interleukin-2 therapy.

28. The method according to claim 22 in which the disease or disorder involving complement is a post-

ischemic reperfusion condition selected from the group consisting of myocardial infarction, balloon angioplasty, post-pump syndrome in cardiopulmonary bypass or renal bypass, hemodialysis and renal ischemia.

29. A method for treating a lung disease or lung disorder involving complement comprising pulmonary administration of an amount of a complement inhibitory protein effective to inhibit complement activity to a subject suffering from the lung disease or lung disorder involving complement.

30. The method according to claim 29 in which the complement inhibitory protein is complement receptor 1, or a fragment, derivative or analog thereof.

31. The method according to claim 30 in which the complement receptor 1 is soluble complement receptor 1.

32. The method according to claim 31 in which the soluble complement receptor 1 has the characteristics of the protein expressed by a Chinese hamster ovary cell DUX B11 carrying plasmid pBSCR1c/pTCSgpt as deposited with the ATCC and assigned accession number CRL 10052.

33. The method according to claim 29 in which the lung disease or lung disorder involving complement in which the disease is selected from the group consisting of dyspnea, hemoptysis, asthma,
chronic obstructive pulmonary disease (COPD), emphysema, and pulmonary embolisms and infarcts.

34. The method according to claim 29 in which the lung disease or lung disorder involving complement is

selected from the group consisting of pneumonia, fibrinogenic dust diseases, pulmonary fibrosis, organic dust diseases, exposure to irritant gasses and chemicals, hypersensitivity pneumonia, parasitic disease, Goodpasture's Syndrome, adult respiratory distress syndrome (ARDS) and pulmonary vasculitis.

35. The method according to claim 34 in which the fibrinogenic dust disease results from exposure to an dust or minerals selected from the group consisting of silicon, coal dust, beryllium, and asbestos.

36. The method according to claim 34 in which the exposure to irritant gasses or chemicals is exposure to a gas or chemical selected from the group consisting of chlorine, phosgene, sulfur dioxide, hydrogen sulfide, nitrogen dioxide, ammonia and hydrochloric acid.

37. The method according to claim 29 in which the lung disease or lung disorder involving complement is bronchoconstriction.

38. The method according to claim 29 in which the lung disease or lung disorder involving complement results from a thermal injury to the lung.

39. The method according to claim 29 in which the lung disease or lung disorder involving complement results from a smoke inhalation injury to the lung.

40. A method for treating bronchoconstriction comprising administering an amount of a complement inhibitory protein effective to inhibit complement activity to a subject suffering bronchoconstriction.

41. The method according to claim 40 in which the complement inhibitory protein is complement receptor 1, or a fragment, derivative or analog thereof.

42. The method according to claim 41 which the complement receptor 1 is soluble complement receptor 1.

43. The method according to claim 42 in which the soluble complement receptor 1 has the characteristics of the protein expressed by a Chinese hamster ovary cell DUX B11 carrying plasmid pBSCR1c/pTCSgpt as deposited with the ATCC and assigned accession number CRL 10052.

44. The method according to claim 40 in which the administration is parenteral.

45. The method according to claim 40 in which the administration is pulmonary.

46. The method according to claim 40 in which the bronchoconstriction results from smoke inhalation.

47. A method for treating anaphylaxis or an anaphylactoid reaction or idiopathic anaphylaxis comprising administering an amount of a complement inhibitory protein effective to inhibit complement activity to a subject suffering anaphylaxis.

48. The method according to claim 47 in which the complement inhibitory protein is complement receptor 1, or a fragment, derivative or analog thereof.

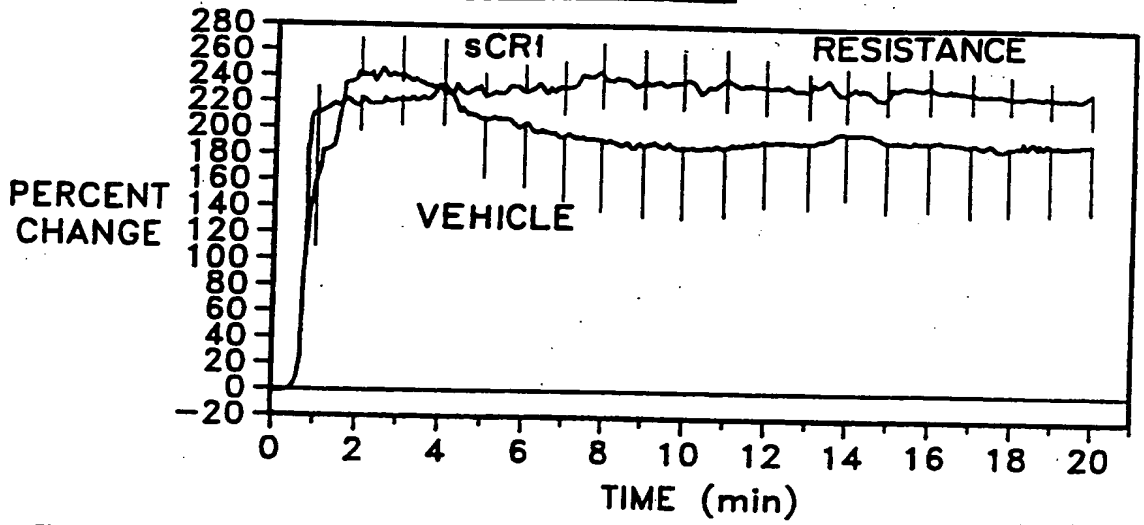
49. The method according to claim 48 which the complement receptor 1 is soluble complement receptor 1.

50. The method according to claim 49 in which the soluble complement receptor 1 has the characteristics of the protein expressed by a Chinese hamster ovary cell DUX B11 carrying plasmid pBSCR1c/pTCSgpt as deposited with the ATCC and assigned accession number CRL 10052.

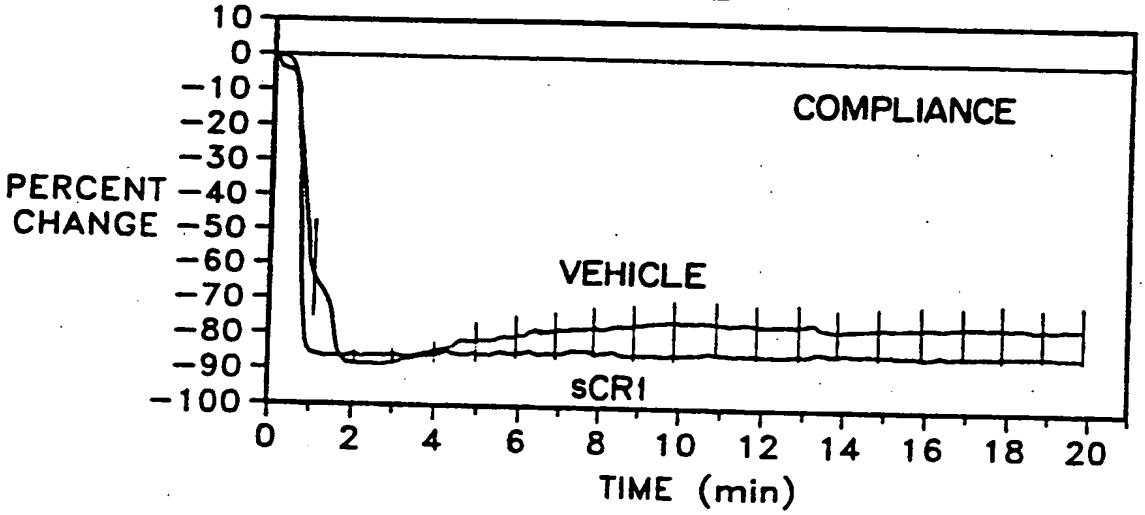
51. The method according to claim 47 in which the administration is parenteral.

52. The method according to claim 47 in which the administration is pulmonary.

FIG—1A PASSIVELY SENSITIZED



FIG—1B PASSIVELY SENSITIZED



FIG—1C PASSIVELY SENSITIZED

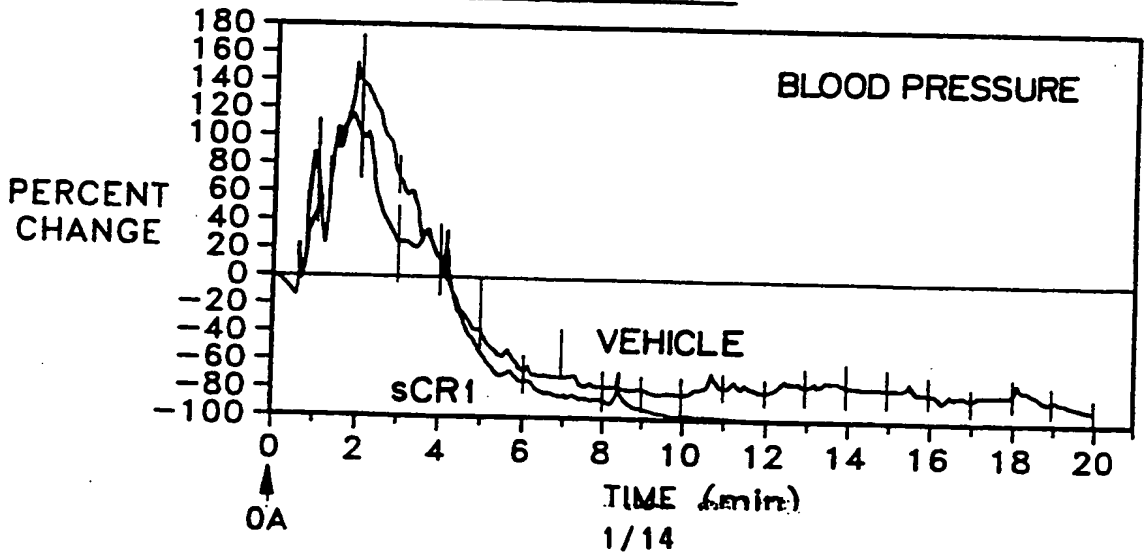


FIG-1D ACTIVELY SENSITIZED

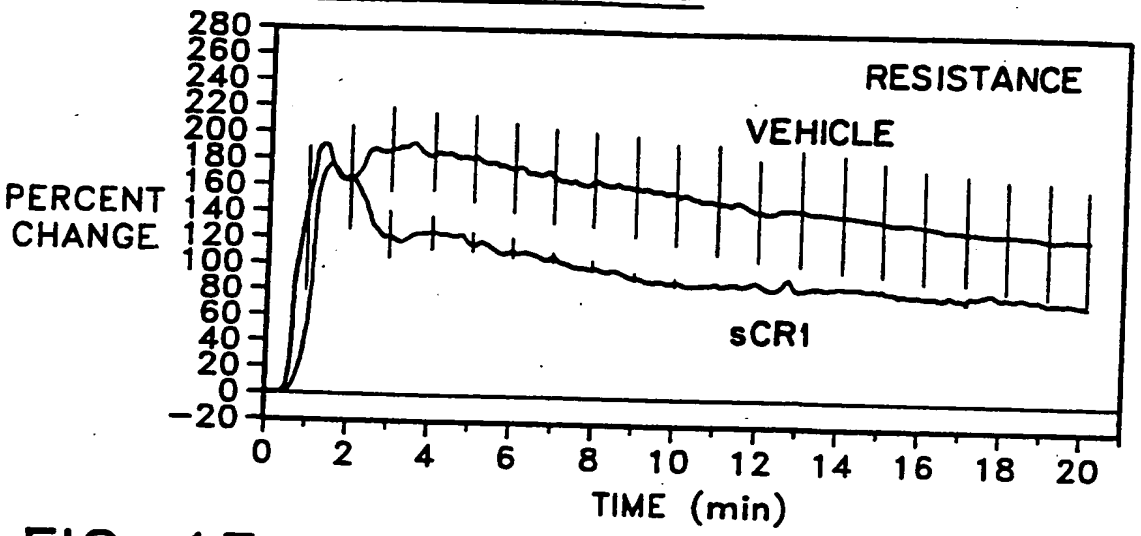


FIG-1E ACTIVELY SENSITIZED

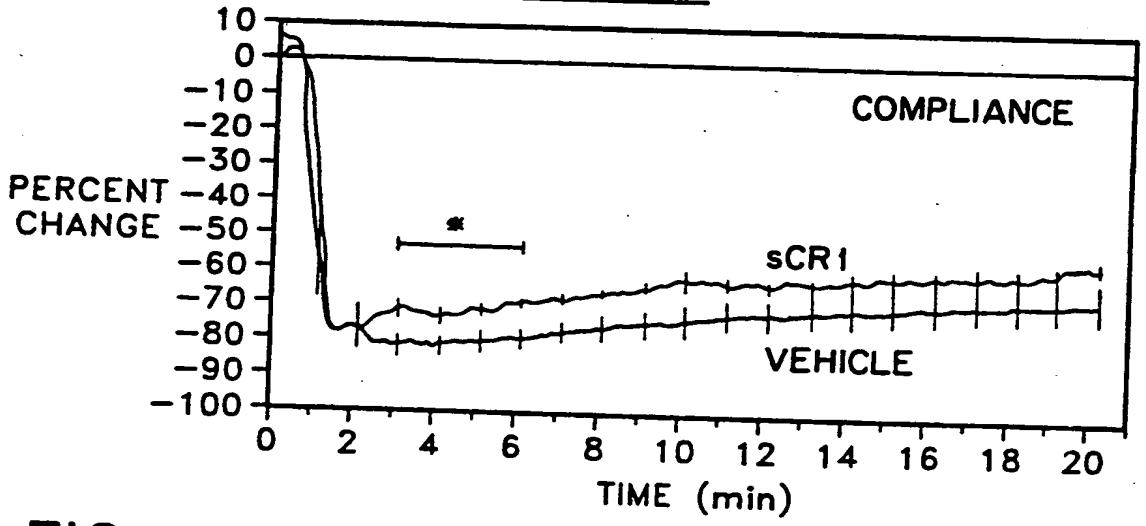


FIG-1F ACTIVELY SENSITIZED

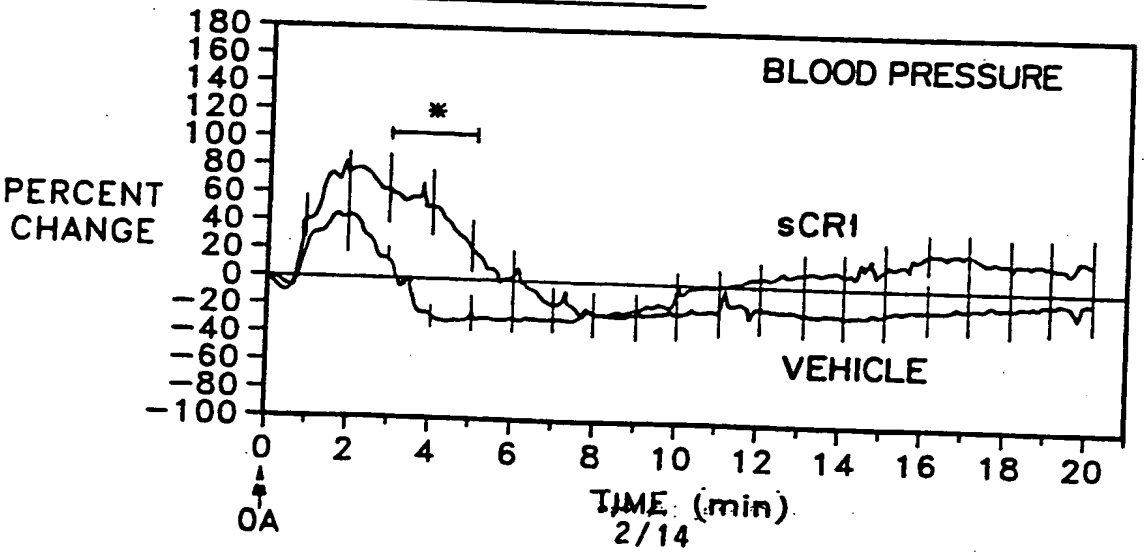


FIG-2A PASSIVELY SENSITIZED

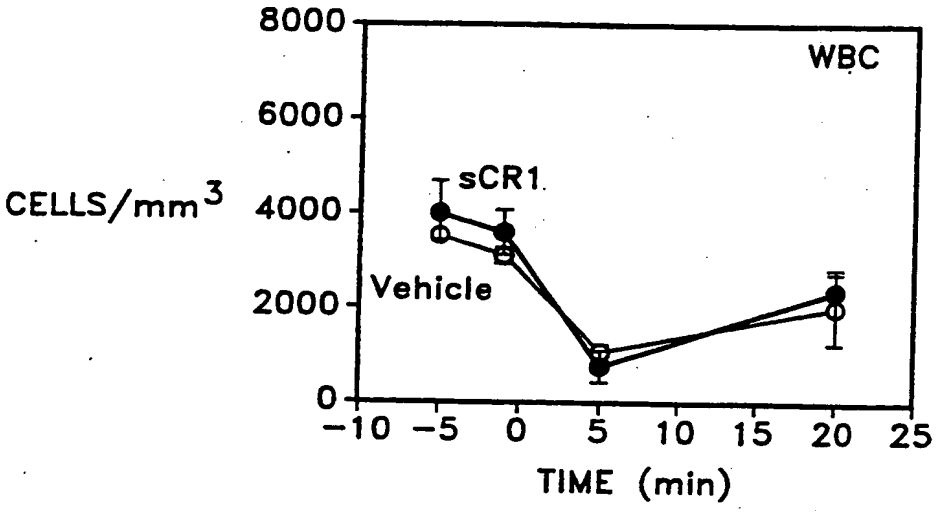


FIG-2B PASSIVELY SENSITIZED

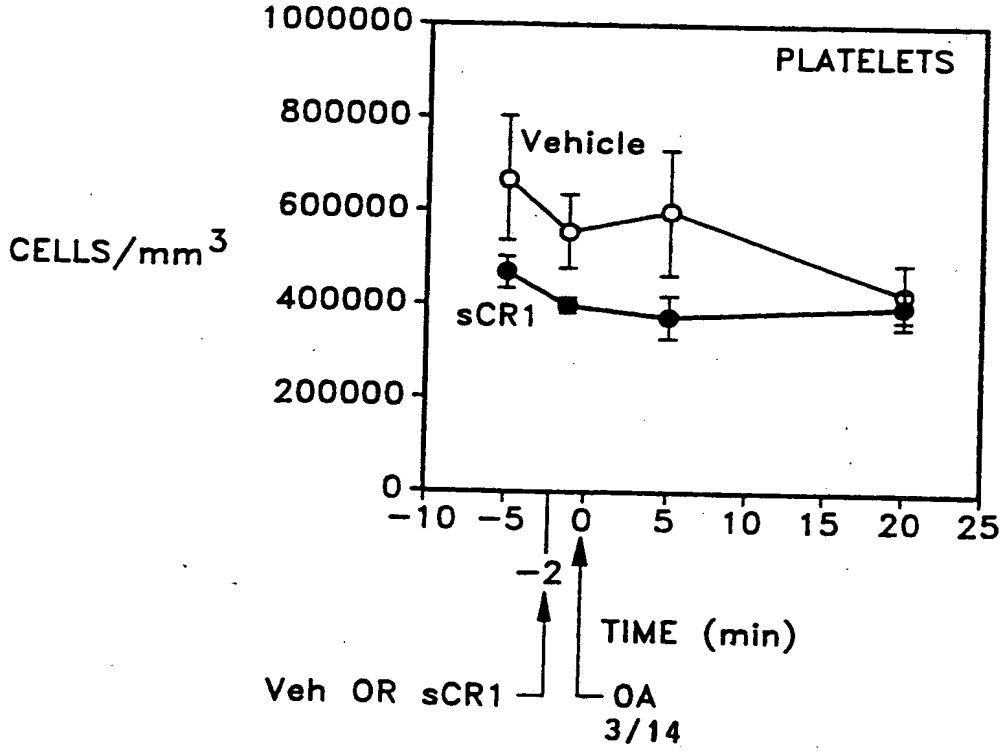


FIG-2C ACTIVELY SENSITIZED

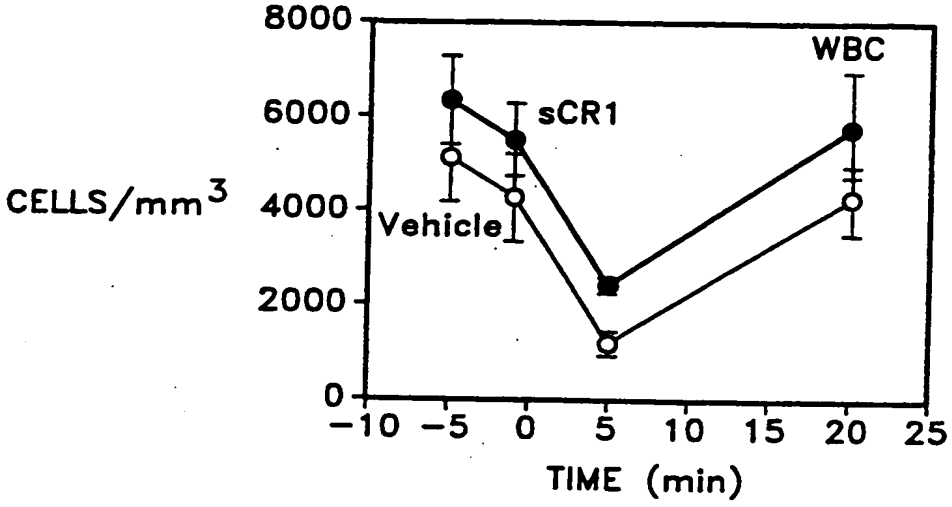


FIG-2D ACTIVELY SENSITIZED

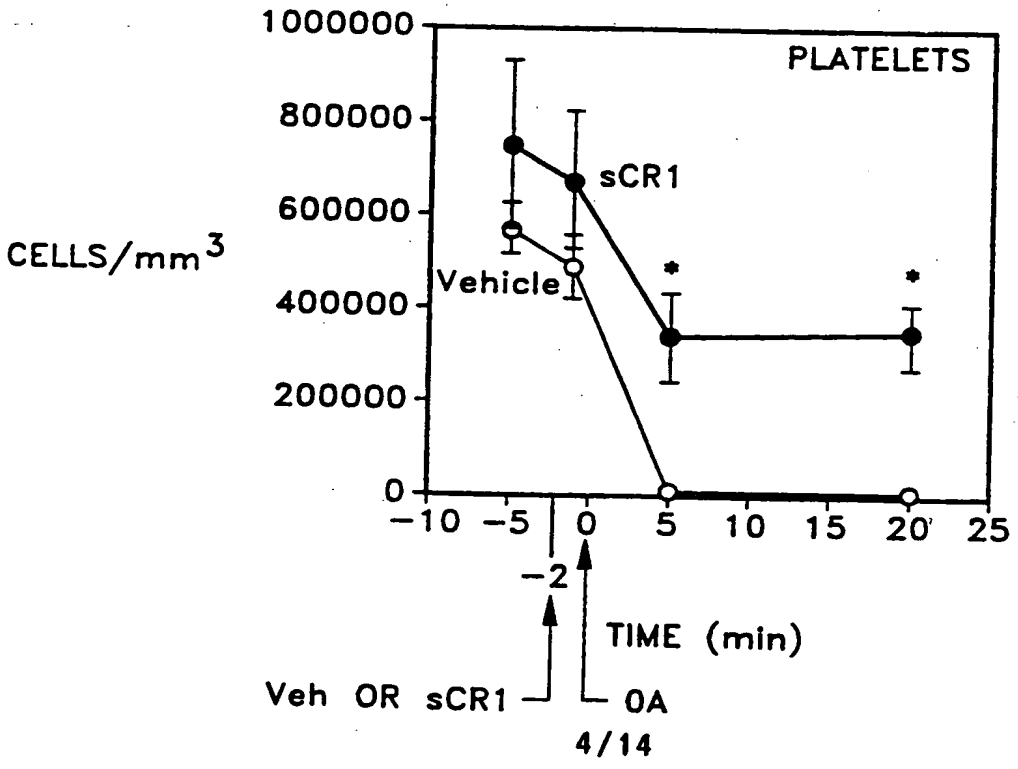


FIG-3A

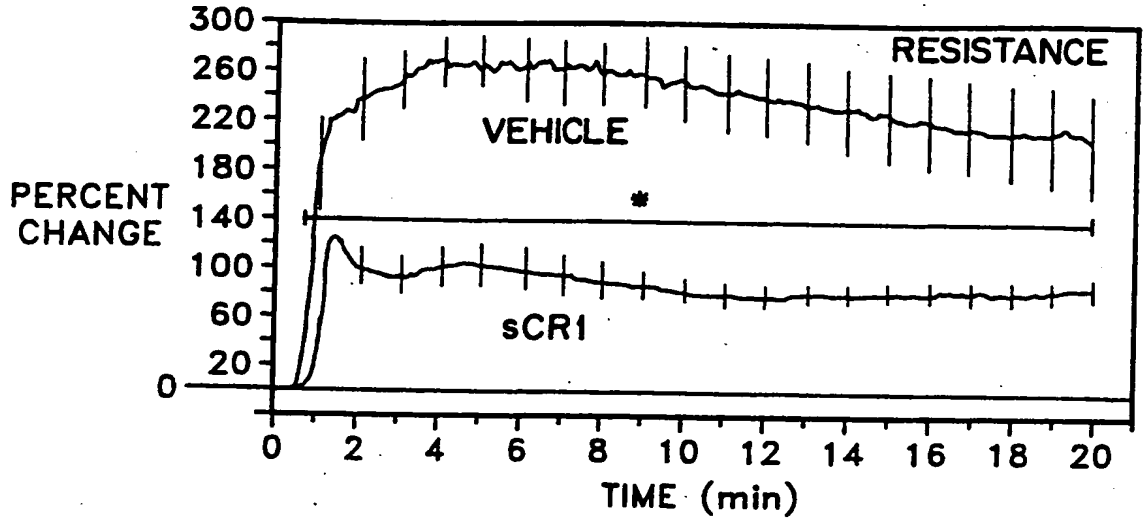


FIG-3B

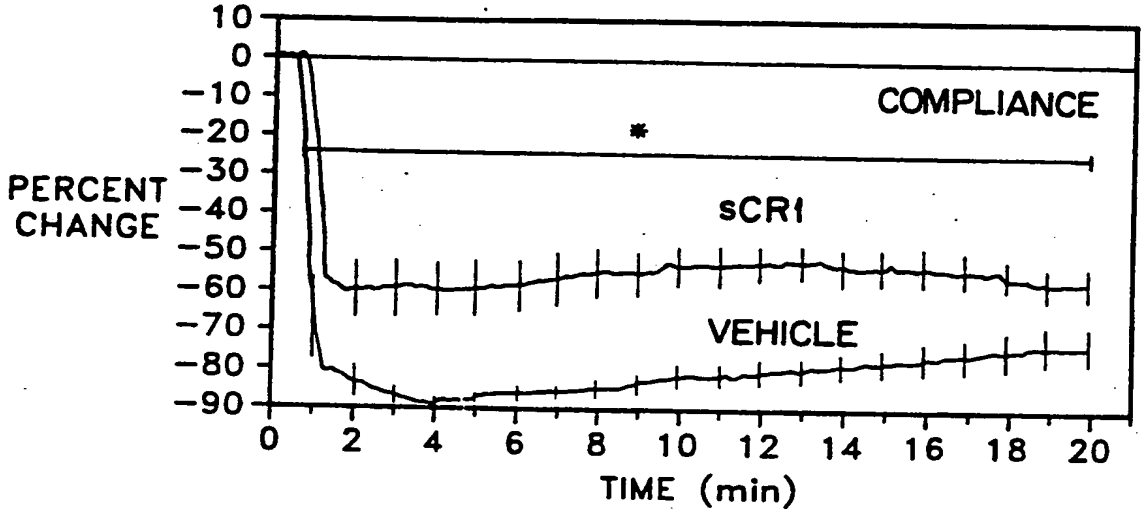
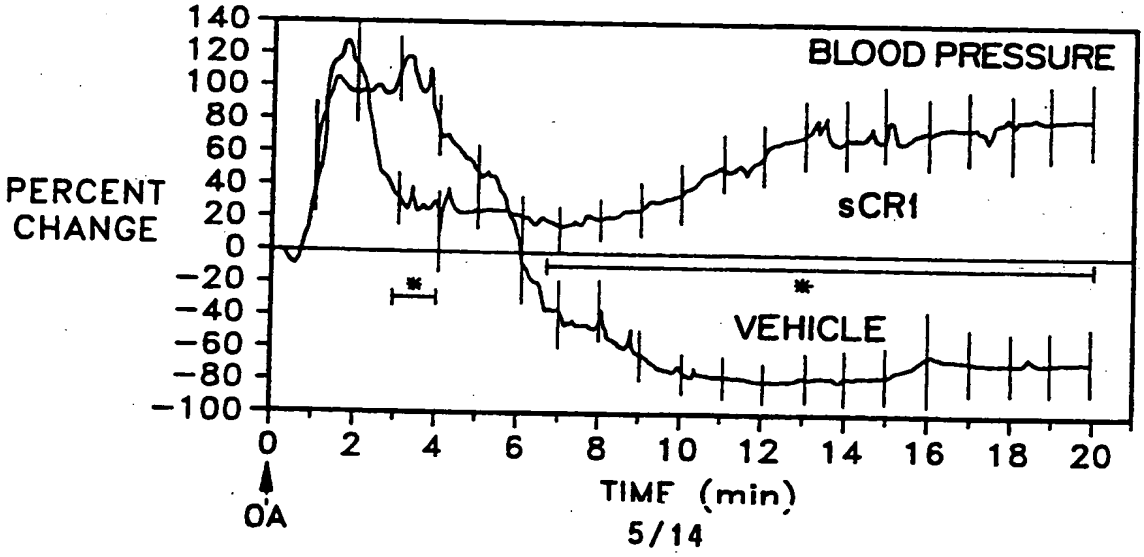


FIG-3C



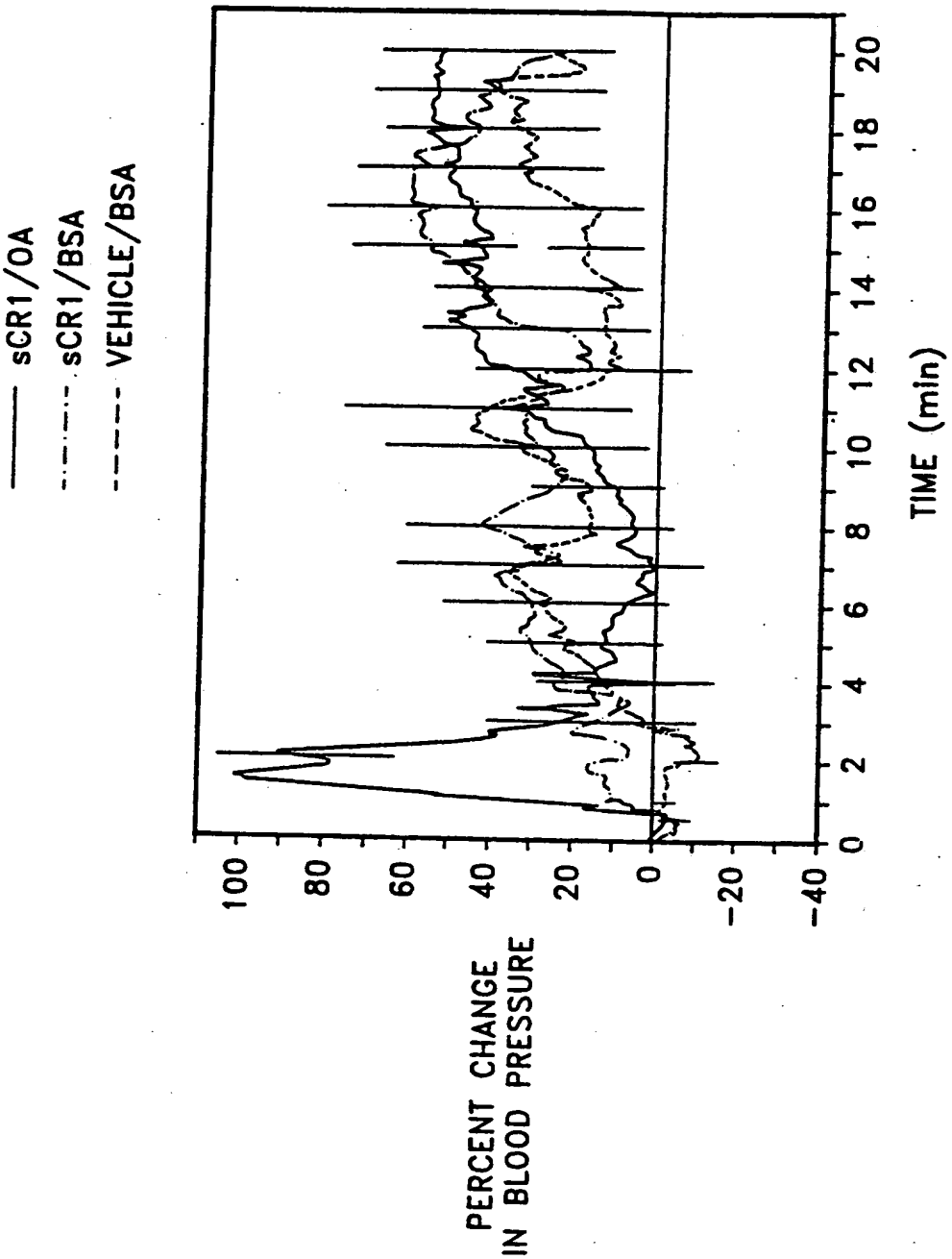

sCR1/OA
sCR1/BSA
VEHICLE/BSA
PERCENT CHANGE IN BLOOD PRESSURE
100
80
60
40
20
0
-20
-40
0
2
4
6
8
10
12
14
16
18
20
TIME (min)

FIG-4

FIG-5A

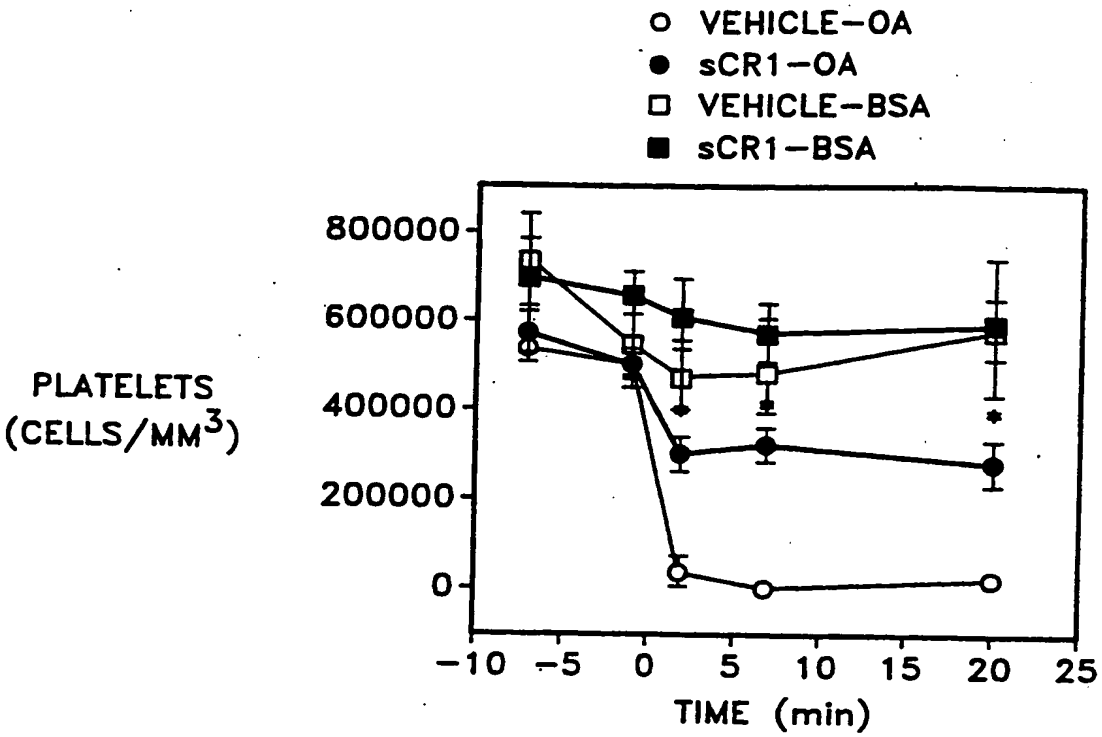

VEHICLE-OA
sCR1-OA
VEHICLE-BSA
sCR1-BSA
PLATELETS (CELLS/MM3)
800000
600000
400000
200000
0
-10 -5 0 5 10 15 20 25
TIME (min)

FIG-5B

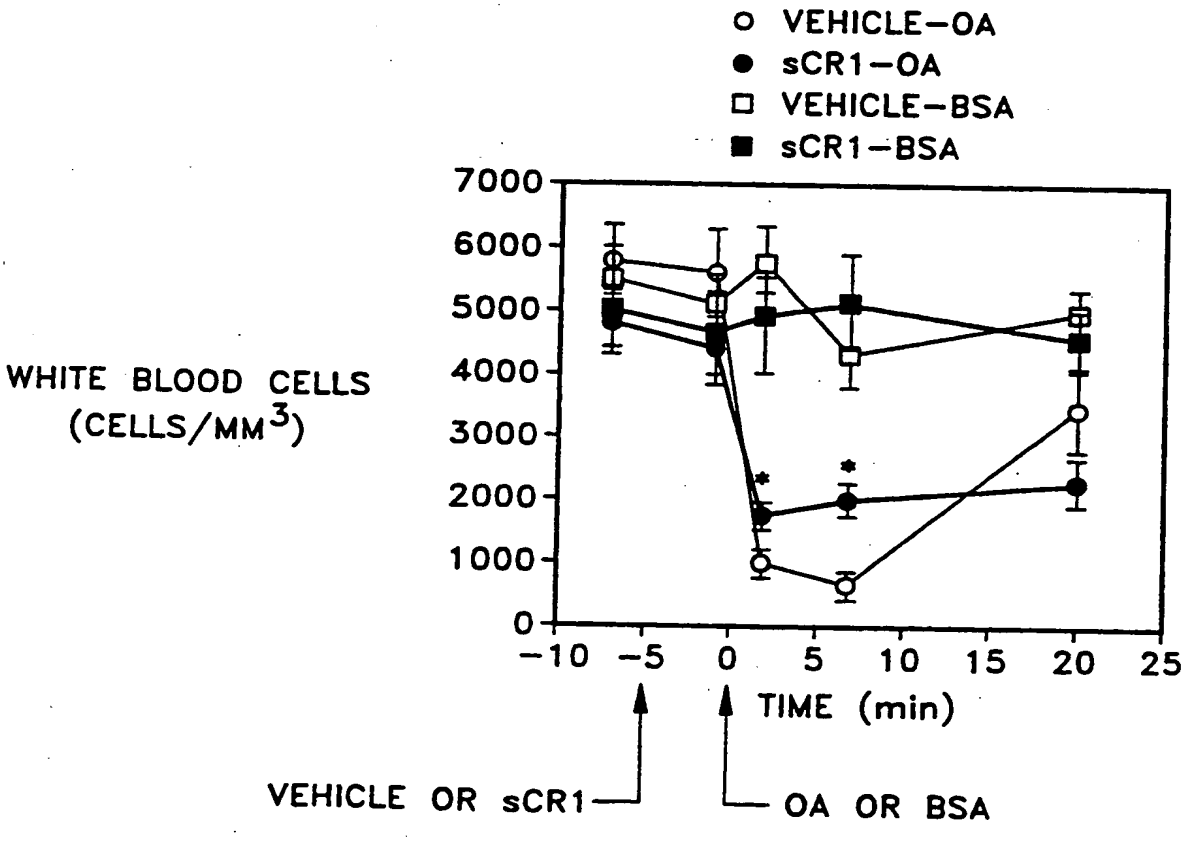

VEHICLE-OA
sCR1-OA
VEHICLE-BSA
sCR1-BSA
WHITE BLOOD CELLS (CELLS/MM3)
7000
6000
5000
4000
3000
2000
1000
0
-10 -5 0 5 10 15 20 25
TIME (min)
VEHICLE OR sCR1
OA OR BSA

FIG-6B RESISTANCE

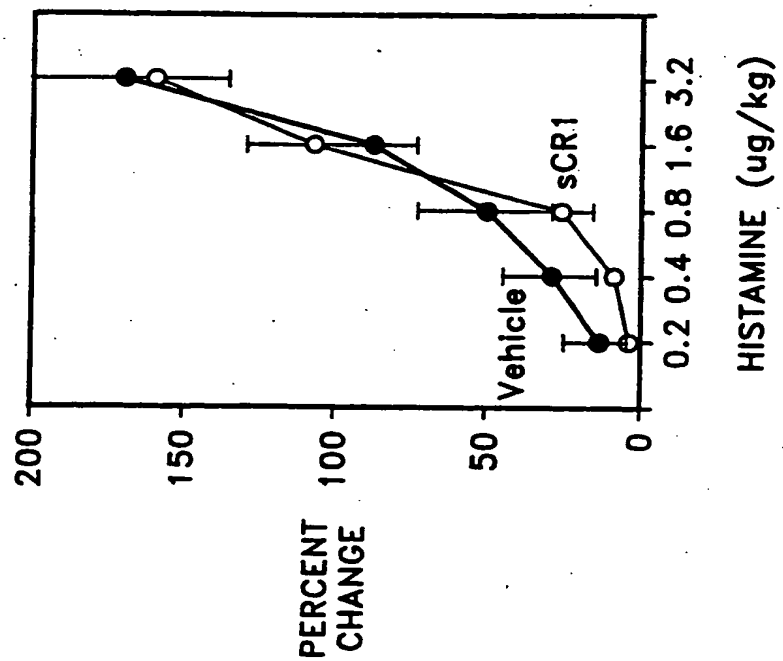
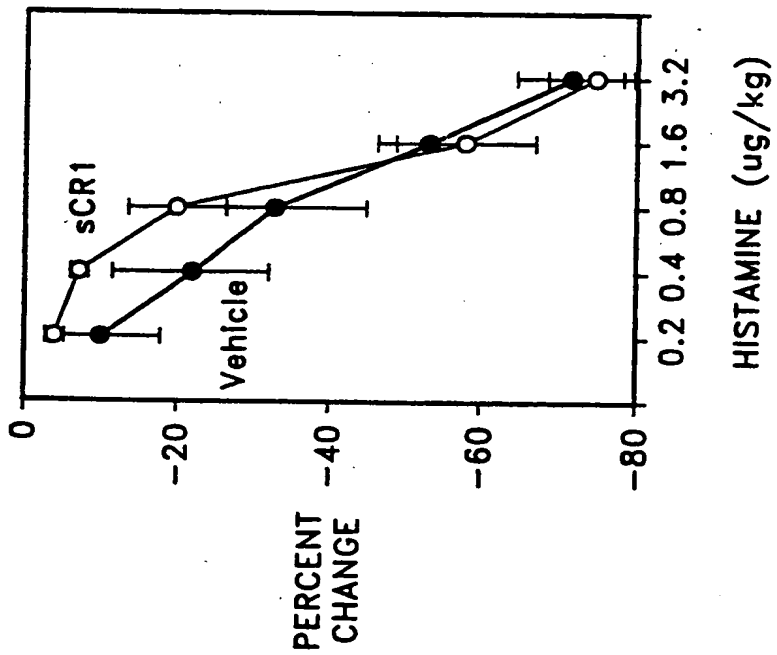
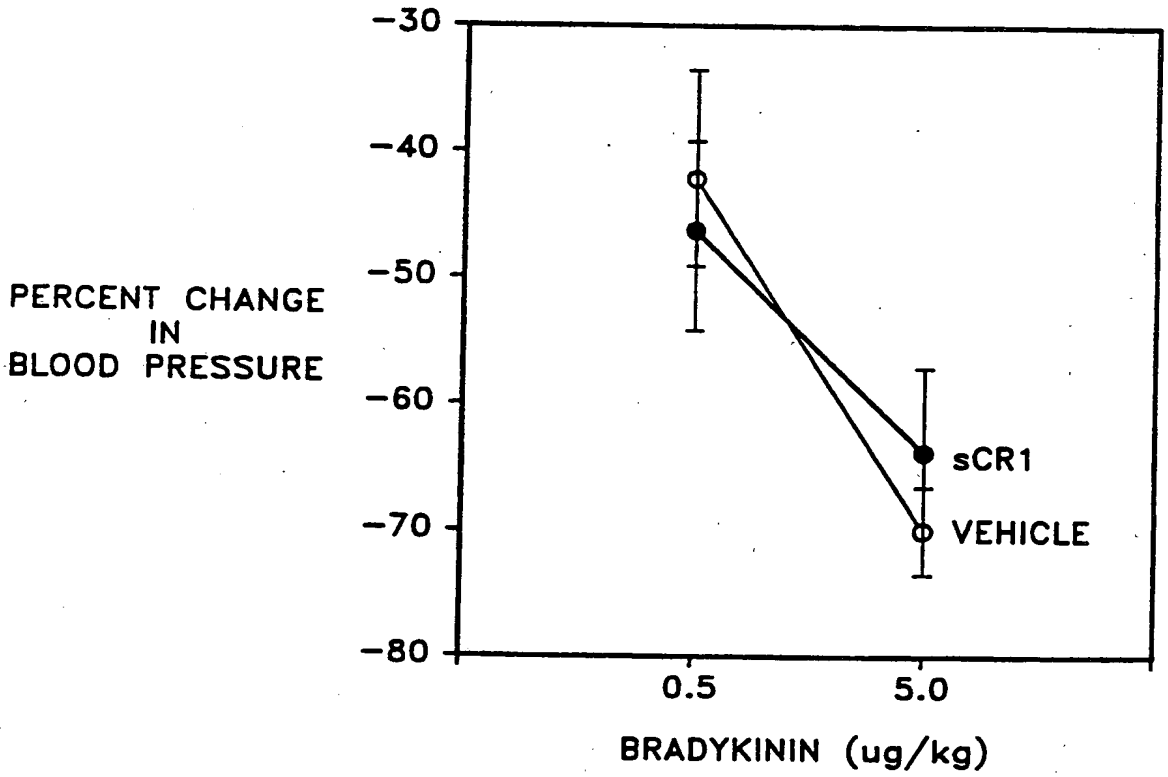


FIG-6A COMPLIANCE



FIG—7


PERCENT CHANGE
IN
BLOOD PRESSURE
−30
−40
−50
−60
−70
−80
0.5
5.0
BRADYKININ (ug/kg)
sCR1
VEHICLE

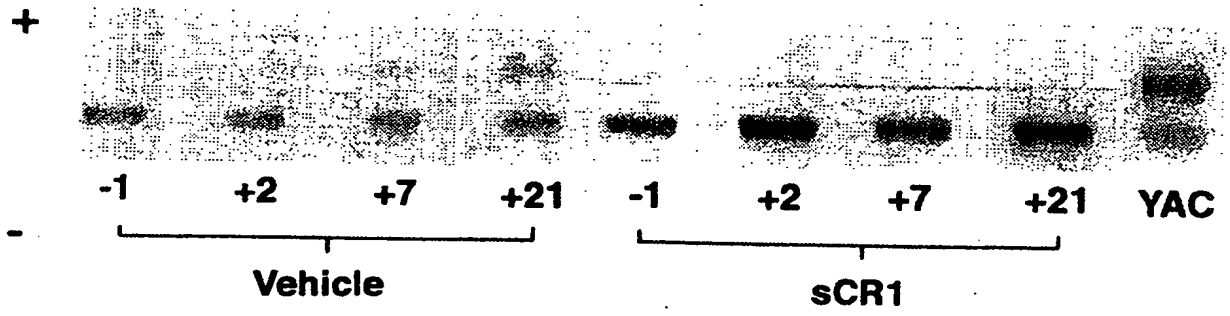
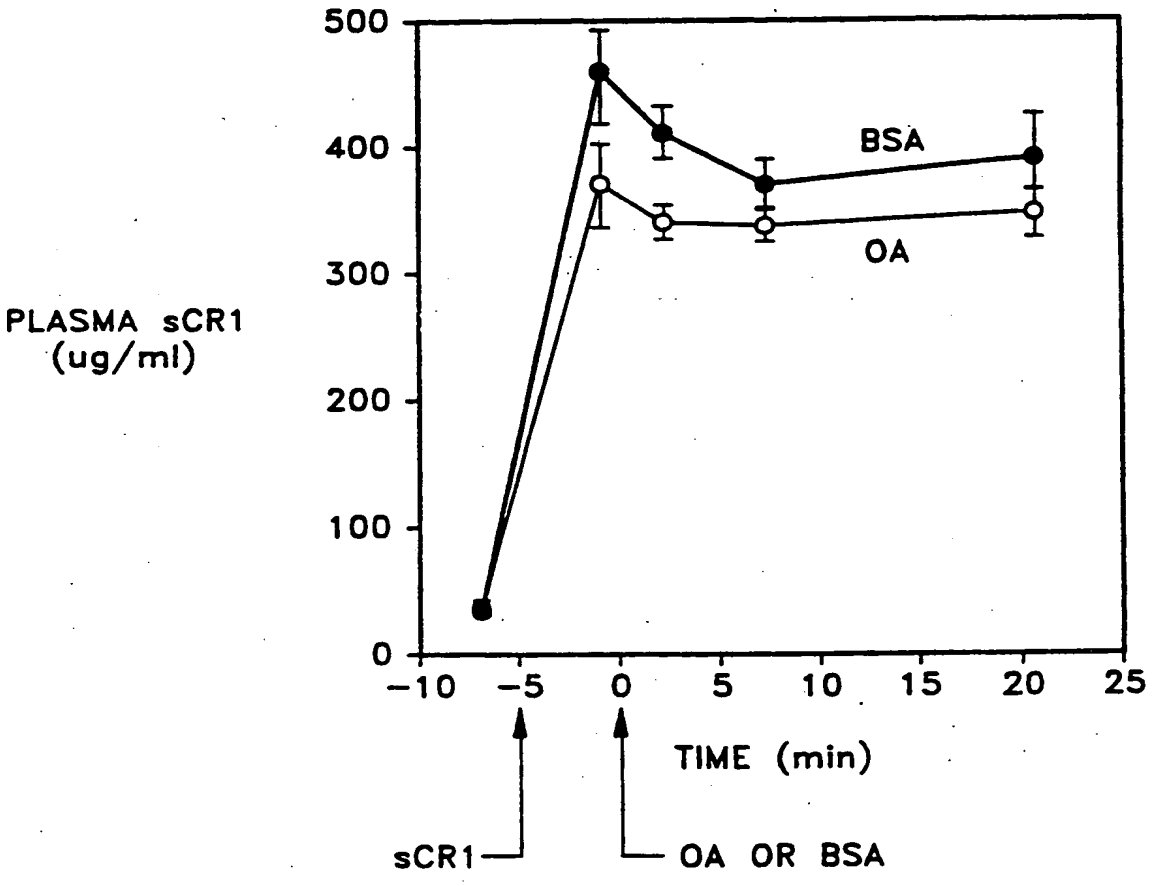


FIG. 8

FIG—9


PLASMA sCR1
(ug/ml)
500
400
300
200
100
0
BSA
OA
−10
−5
0
5
10
15
20
25
TIME (min)
sCR1
OA OR BSA

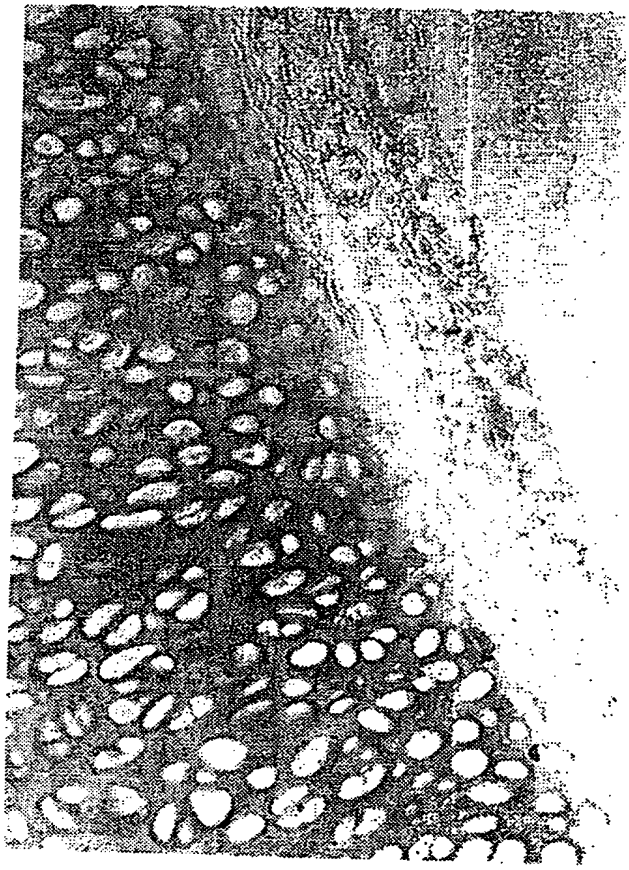


FIG. 10A

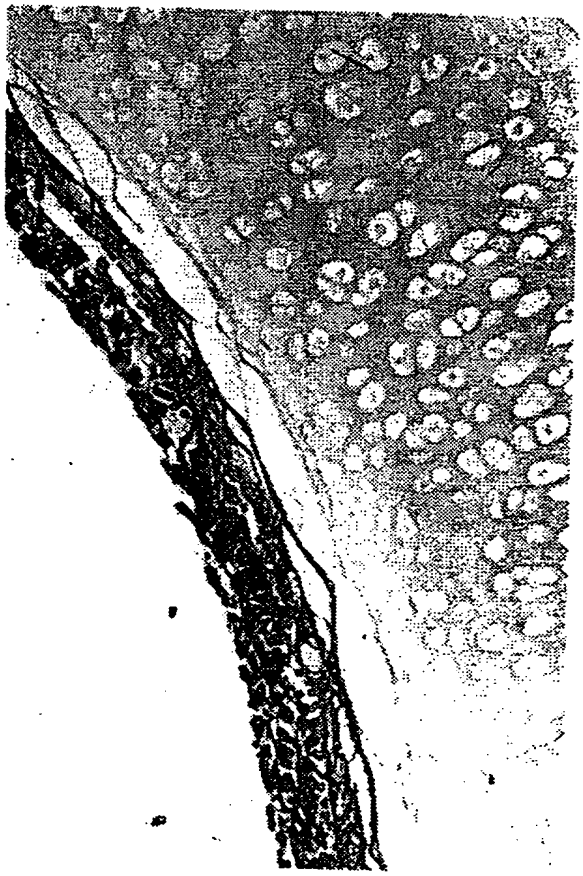


FIG. 10B

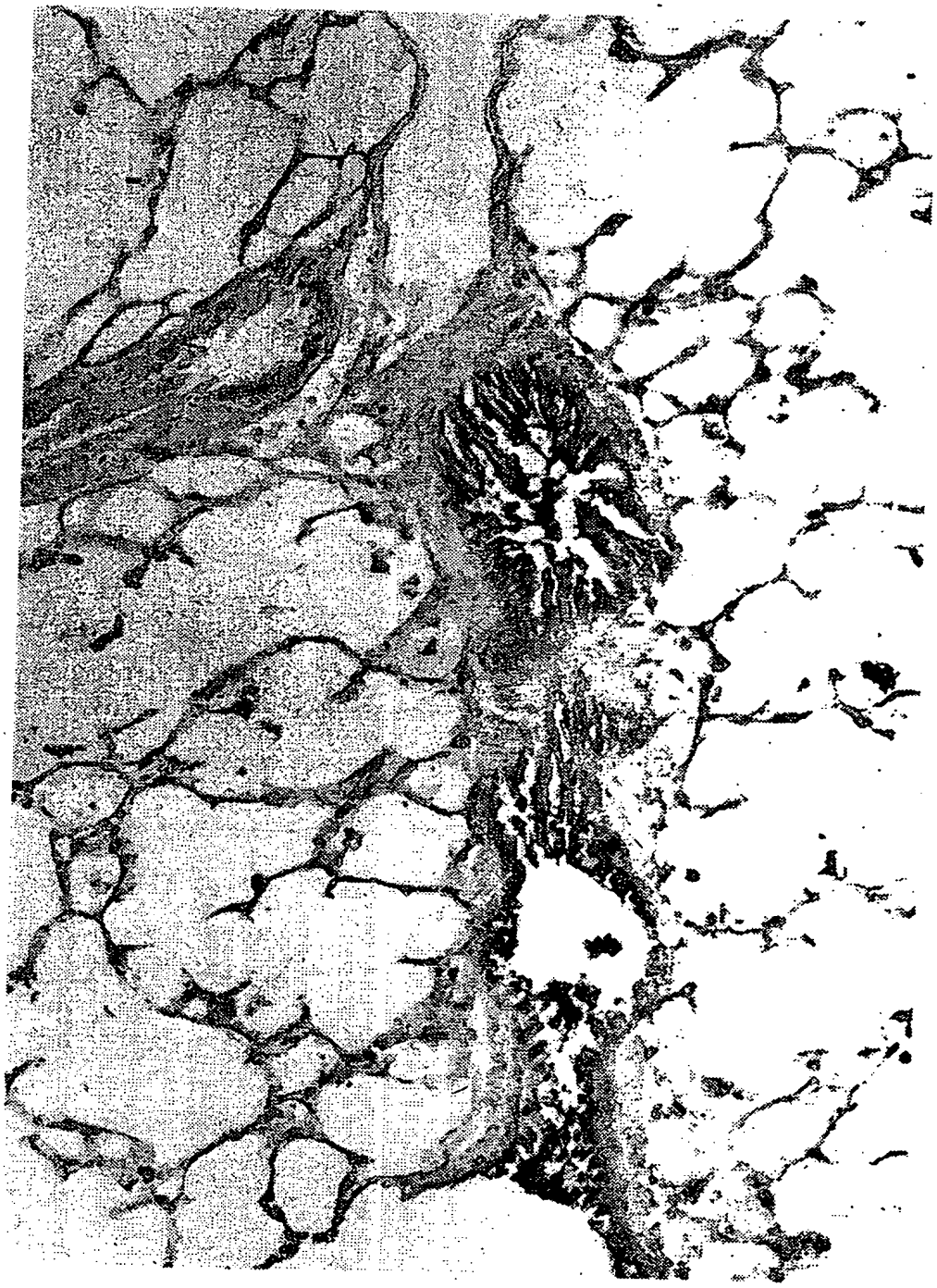


FIG. 11A

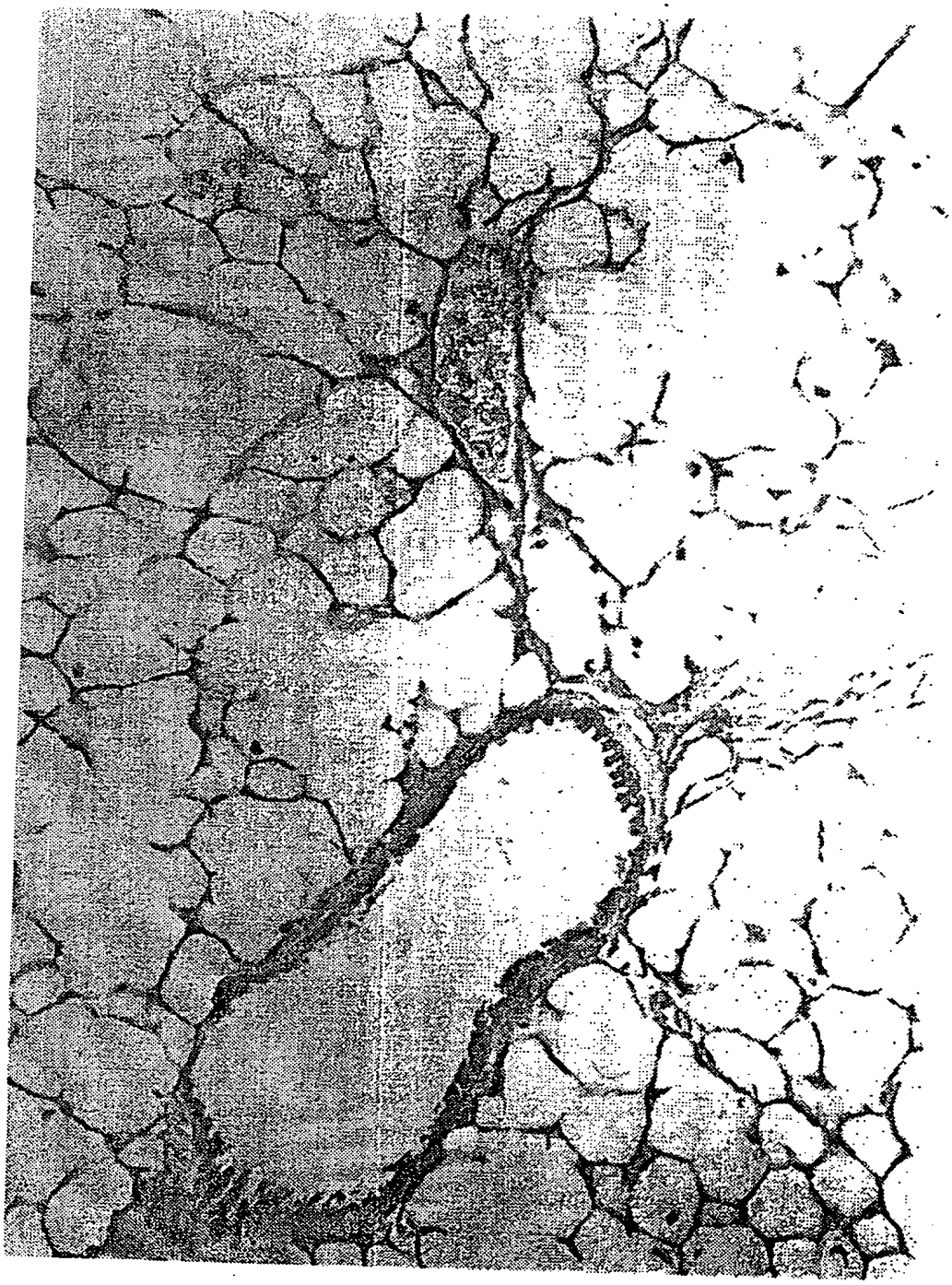


FIG. 11B

INTERNATIONAL SEARCH REPORT

In. ational application No.
PCT/US94/01405

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :A61K 37/64, 9/72
US CL :514/2, 8; 424/499
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/2, 8; 424/499

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO, A1, 89/09220 (FEARON et al.) 05 October 1989, especially pages 1-8 and the claims.	1-4, 16, 17 --- 5-15, 18-52
Y	Klinische Wochenschrift, Volume 69, issued 1991, P. A. Ward et al., "New Insights into Mechanisms of Oxyradical and Neutrophil Mediated Lung Injury", pages 1009-1011, see the entire document.	1.52
Y	Clinical and Experimental Immunology, Volume 86, Supplement 1, issued October 1991, D. T. Fearon, "Anti-inflammatory and immunosuppressive effects of recombinant soluble complement receptors", pages 43-46, see the entire document.	1-52

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
13 MAY 1994

Date of mailing of the international search report
MAY 23 1994

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